MARKED UP VERSION OF THE CLAIMS

21. (cancelled).

22. (cancelled).

23. (cancelled).

24. (cancelled).

25. (cancelled).

26. (cancelled).

27. (cancelled).

28. (cancelled).

29. (cancelled).

30. (cancelled)..

31. (currently amended) A method for treating a strabismus, the method comprising the step of administering to a human patient a therapeutically effective amount of botulinum toxin type B to treat strabismus, wherein the botulinum toxin type B is administered by intramuscular injection or by subcutaneous injection, and the administration of the botulinum toxin type B results in alleviation of ~~a muscle spasm symptom of~~ the strabismus within 1 day to ~~about~~ 7 days.

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(57) Abstract

The present invention provides a method for relieving pain, associated with muscle contractions, a composition and a method of treating conditions such as cholinergic controlled secretions including excessive sweating, lacrimation and mucus secretions and a method for treating smooth muscle disorders including, but not limited to, spasms in the sphincter of the cardiovascular arteriole, gastrointestinal system, urinary, gall bladder and rectum, which method comprises administering to the patient suffering from said disorder or condition a therapeutically effective amount of Botulinum toxin selected from the group consisting of Botulinum toxin types B, C, D, E, F and G.

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INTERNATIONAL SEARCH REPORT

Interna ! Application No
PCT/US 94/14717

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EXPERIENTIA, vol.33, no.6, 15 June 1977 pages 750 - 751 KONDO T., ET AL. 'Modification of the action of pentagastrin on acid secretion by botulinum toxin' * see the whole document * ---	1
Y	SCHWEIZ. MED. WSCHR., vol.104, pages 685 - 693 G. JENZER ET AL. 'Botulismus Typ B' * see the summary, Page 690, Figure 6 and left column, ultimate paragraph * --- -/--	1

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
27 April 1995	15. 09. 95

Name and mailing address of the ISA	Authorized officer
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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/US 94/14717

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NEW SCIENTIST, no.1746, 8 December 1990 page 24 N. HENESON 'Deadly toxin calms excited muscles' * see the whole article * ---	1
A	ARCH. OPHTHALMOL., vol.103, 5 pages 1305 - 1306 SAVINO P.J., ET AL 'hemifacial spasm treated with botulinum A toxin injection' * see the abstract * ---	1
A	EUR. NEUROL., vol.33, pages 199 - 203 D. BOGHEN ET AL. 'Effectiveness of Botulinum toxin in the treatment of spasmodic torticollis' * see the abstract * -----	1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

In. .iational application No.
PCT/US 94/ 14717

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- claims 1-5
- claims 6-13
- claims 14-20

- See (1) additional sheet PCT/ISA/210

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-5

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US94/14717

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

LACK OF UNITY OF INVENTION

1. Claims: 1-5 Method for treating cholinergic secretions using Botulinum toxin

2. Claims: 6-13 Method for treating smooth muscle disorders and pain associated therewith using Botulinum toxin

3. Claims: 14-20 Method for treating spastic muscle disorders and pain associated therewith using Botulinum toxin

The present application lacks unity of invention since it describes 3 different subjects defined below which are not linked by a common novel and inventive concept.

The separate inventions/groups of invention are:

A.) Claims 1-5 Method for treating cholinergic secretions using Botulinum toxin

B.) Claims 6-13 Method for treating smooth muscle disorders and pain associated therewith using Botulinum toxin

C.) Claims 14-20 Method for treating spastic muscle disorders and pain associated therewith using Botulinum toxin

(See also page 4 line 29 - page 5 line 7 of the application.)

It is to be noted the use of Botulinum toxins for treating diseases, especially those included in the above groups B) and C) is known as acknowledged in the description at pages 1-3. See also ~~D. Boghen~~ and M. Flanders, Eur. Neurol., 1993, Vol. 33, p. 199-203, which describes the effectiveness of Botulinum toxin in the treatment of spasmodic torticollis and associated pain.

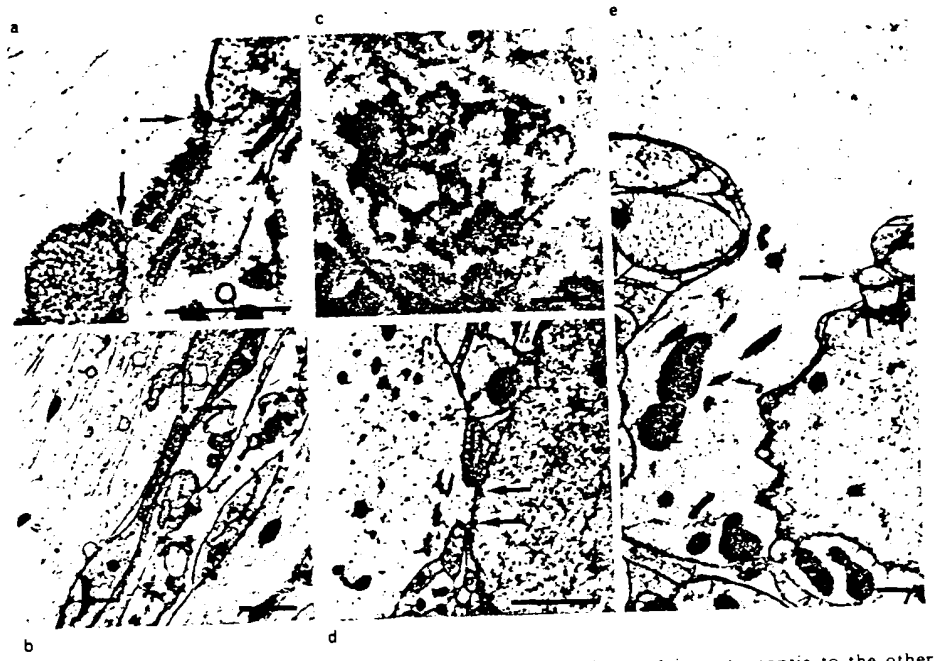

Fig. 2. *a* A giant interneuron is presynaptic to 2 small blebs. 2 divergent diadic synapses are arrowed. The blebs are packed with synaptic vesicles and are themselves presynaptic as well as postsynaptic structures. A transverse section taken at the base of the median ocellar nerve. Bar 0.25 μm indicates magnification. – *b* A number of long blebbed dendrites are presynaptic to giant interneurons which are themselves presynaptic to other small profiles in the same area. 2 divergent diads are arrowed. A longitudinal section from the median ocellar nerve proximal to the brain. Bar 0.5 μm. – *c* A 10 μm wax section through the lateral ocellar tract showing profiles filled by cobalt diffusion from the ventral nerve cord and treated by the modified Timm technique. Long, blebbed dendrites ring the giant interneurons over a considerable length. Thick sections indicate that a number of intrinsic interneurons terminate in a similar way in this region. Bar 10 μm. – *d* 2 giant interneurons are directly apposed over a short length of their membrane, 1 is postsynaptic to the other and also postsynaptic to a smaller profile. In this area a large interneuron may be both pre- and postsynaptic. Input or output synapses are usually grouped together in discrete areas a few microns apart. Here also synapses are usually diadic and divergent. Transverse section of the lateral ocellar nerve in the pars intercerebralis. Bar 1 μm. – *e* The giant interneurons give off very short, stubby collaterals which wrap around other giant second order cells. Small dendrites from third order cells interdigitate repeatedly between them. Synaptic arrangements are generally complex, diadic and reciprocal, black arrows. A transverse section in the brain anterior to the protocerebral bridge. Bar 0.25 μm.

Integration by means of slow potentials in second order cells is a common feature of visual sensory neuropile[10]. In the insect compound eye the large monopolar cells of the lamina only respond to retinal stimulation with graded hyperpolarisations[5,11]. A unique feature in the ocellar system is the size of the second order cells and the distances involved and the fact that the ocellar neuropilar area has become extended over the axonal length of the fibre. A contributory factor here may be the fact that the lateral and median ocelli are linked in pairs by 2 large axons. The axonal integration area described may thus in effect form a common neuropile area for each pair of ocelli. Information processing along visual interneurons of this size and accessibility offers a most promising preparation for examining mechanisms of graded synaptic transmission.

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Modification of the action of pentagastrin on acid secretion by botulinum toxin[1]

T. Kondo[2] and D. F. Magee

Department of Physiology, Creighton University, School of Medicine, 2500 California Street, Omaha (Nebraska 68178/USA), 26 November 1976

Summary. I.v. botulinum toxin after 60–90 min abolished the dose-response relationship between pentagastrin and gastric acid secretion in anesthetized rats and guinea-pigs. The toxin reduced but did not abolish the acid stimulatory effect of histamine. As expected, the acid response to vagal stimulation was abolished and that to methacholine in rats was unaltered by the toxin.

Vizi et al.[3] have provided evidence that pentagastrin(PG) does not act directly to stimulate guinea-pig intestinal muscle, but via a cholinergic intermediary mechanism. We have found[4,5] that after morphine sulphate or hemicholinium administration to conscious Heidenhain pouch dogs a positive dose-response relationship between i.v. pentagastrin and gastric acid secretion is no longer obtainable. Morphine depresses acetylcholine release at cholinergic neuroeffector sites[6] and hemicholinium interferes with acetylcholine synthesis[7]. This suggests that the action of PG on gastric secretion also requires cholinergic mediation.

Material and methods. As a final test of this hypothesis we have measured PG-stimulated gastric secretion in anesthetized (chloralose) guinea-pigs and rats before and after botulinum toxin. In each animal after anesthesia

the external jugular vein was cannulated and a tracheal tube inserted. A stomach tube was passed and the pylorus cannulated. After completing the preparation of the animal i.v. saline was given for 30 min (3 collections), then 0.04 μg/min pentagastrin until a plateau of acid secretion was reached. At this point 0.5 ng/100 gm of botulinum toxin was given as an i.v. bolus in phosphate buffer at pH 6.8. Pentagastrin was continued for 4 more 10-min periods at 0.04 μg/min. Then it was increased to 0.1, 0.2 and 0.4 for 2 collections at each level. This was succeeded by pentagastrin at a baseline level of 0.1 μg/min for the remainder of the experiment plus histamine, 4 μg/min in guinea-pigs, 0.5 μg/min of methacholine in rats and bilateral vagal stimulation for 20 min in both (2 collections). Before histamine the animals were given 2 mg of promethazine HCl i.v.

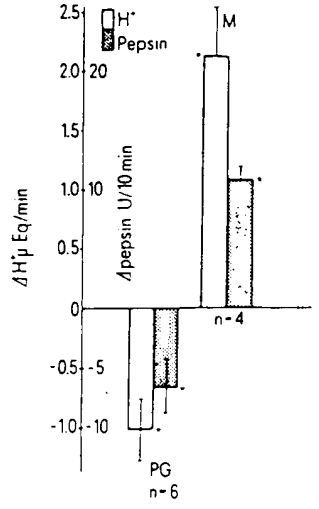

Fig. 1. The effect ± SE of botulinum toxin on pentagastrin-(PG) stimulated acid and pepsin secretion in rats and the effect of methacholine (M) after botulium toxin. PG was given throughout. The bars represent difference from PG baseline (0 line) in the same animal. *significant change from PG baseline, $p < 0.05$. n = number of animals.

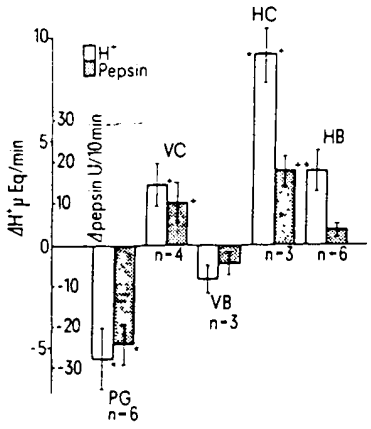

Fig. 2. The effect ± SE of botulinum toxin on pentagastrin-stimulated acid and pepsin secretion and the effect of vagal stimulation and histamine in control guinea-pigs (VC and HC) and in guinea-pigs after botulinum toxin (VB and HB) as in figure 1. PG was given throughout and the bars represent change from the PG baseline (0 line) response in both groups of animals. *significant change from PG baseline, $p < 0.05$. ⁻significant difference from animals given toxin, $p < 0.05$. n = number of animals.

At 10-min intervals the stomachs were flushed with 5 ml of warm saline. These washings were titrated and samples saved for pepsin determination using Anson's[8] hemoglobin method. Controls were conducted in separate animals and duplicated the experiment exactly except that the phosphate buffer did not contain botulinum toxin. Comparison was made both with the change from control in the same animal and collection by collection between control and experimental means using student's t-test for paired and group comparison respectively.

Results. Botulinum toxin brought about a prompt decline to near vanishing point of pentagastrin-stimulated acid and pepsin secretion in rats (figure 1). No subsequent dose response to pentagastrin could be obtained at any of the PG doses tried. Methacholine still produced good secretion despite botulinum toxin. Vagal stimulation was never successful in either control or experimental rats. Substantially the same was seen in guinea-pigs (figure 2). Botulinum toxin abolished the acid and pepsin response to vagal stimulation but not to histamine. Vagal stimulation produced copious secretion in control guinea-pigs. The response to histamine was, however, significantly smaller after toxin than in the control animals. No stimulation of acid and pepsin was obtained with increasing doses of gastrin. The major difference from the rats was in that botulinum toxin itself increased acid secretion in guinea-pigs. This initial burst of secretion gradually declined over 60–90 min. An attractive explanation for this is possible histamine liberation by the toxin in this histamine-sensitive species.

Discussion. Since botulinum toxin exerts its pharmacological action by preventing liberation of acetylcholine from nerve endings, these experiments add further evidence to support the hypothesis that acetylcholine is a necessary intermediate for pentagastrin stimulation of gastric secretion. That the secreting cells are themselves active is evident from the continued efficacy of 2 direct-acting stimulants, histamine and methacholine. Histamine was less effective in guinea-pigs after toxin, but the action of methacholine in rats was unimpaired. Proof that cholinergic secretory mechanisms have indeed been blocked is provided by the failure of vagal stimulation after botulinum toxin to increase acid or pepsin secretion in guinea-pigs. The gastric secretory cholinergic mechanism seems to display a marked sensitivity to botulinum toxin since within the time span of our experiments breathing and skeletal reflex activity remained virtually unimpaired. Similar sensitivity was seen to hemicholinium which, in conscious dogs, produces no obvious impairment other than of gastric secretion[4].

1 Acknowledgment. We are grateful to the generosity of Dr Edward J. Schantz, Food Research Institute, University of Wisconsin, for the botulinum toxin used. – This research was supported by NIH grant 1 RO1 AM 17125, The Secretion of Pepsin.

2 Visiting Instructor from Nagoya University, Nagoya, Japan.

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Schweiz. med. Wschr. 104, 685–693 (1974)

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Botulismus Typ B

Bericht über milde Verlaufsformen mit vorwiegend autonomen Innervationsstörungen

G. Jenzer, M. Mumenthaler, H. P. Ludin und F. Robert

Zusammenfassung. Anhand von 9 eigenen Fällen wird eine leichte Verlaufsform einer Botulinusintoxikation vom Typ B mit autonomen Funktionsstörungen ohne nachweisbare neuromuskuläre Beteiligung dargestellt. Einer Schilderung von Methoden zur objektiven Verlaufsbeobachtung folgen eine Orientierung über pathophysiologische Zusammenhänge sowie Bemerkungen zu Therapie und Differentialdiagnose. Verlaufsbeobachtungen weisen auf eine unerwartet lange Persistenz subjektiv meist nicht mehr wahrnehmbarer Funktionsstörungen von Tränendrüsen und Parotis.

Die vorliegenden Erfahrungen sprechen gegen eine Antitoxinbehandlung in der Spätphase eines in Remission begriffenen, leichten Botulismus. Der Einsatz von Guanidin kann demgegenüber als verhältnismässig betrachtet werden.

In Korrelation mit Angaben aus der Literatur wird auf die Möglichkeit einer besonderen Affinität des B-Toxins für die autonome Innervation beim Menschen hingewiesen.

Summary. 9 cases of human type B botulism with mild course and almost selective effect upon autonomic nervous functions are described. After clinical remission a reduction of lacrimal and salivary secretions could be demonstrated for up to several months. Our experience argues against the use of antitoxin in the late course of such cases with minor symptomatology but in favour of guanidine, which offered some subjective improvement. From comparable published cases it seems that autonomic nervous functions in the human are particularly susceptible to B type toxin.

Durch französischen Briekäse wurden im Verlauf einer kleinen Epidemie in der Schweiz im Sommer 1973 wenigstens 48 Personen mit Botulinustoxin Typ B vergiftet. Eingehende Untersuchungen erlaubten eine genaue Rekonstruktion der epidemiologischen Verhältnisse [37]. 9 dieser so betroffenen Patienten, 5 Frauen und 4 Männer mit einem Durchschnittsalter von 31 Jahren, wurden in unserer Klinik untersucht, teils unter kurzfristiger Hospitalisation zur Vornahme weitergehender Untersuchungen.

Fallschilderung. Der Erkrankungsfall eines 42jährigen Kaufmanns ist exemplarisch und soll detailliert dargestellt werden:

D. G. ass am 25. Juli 1973 etwa 100 g des inkriminierten, im Geschmack angeblich nicht beeinträchtigten Käses. 12 Std. später machte sich ein Völlegefühl mit Obstipation bemerkbar. Nach 2–3 Tagen fand sich der Patient ungewohnt antriebslos, bei der Arbeit ineffizient und gedankenverloren. Mittlerweile hatte sich ein allgemeines Schwächegefühl entwickelt sowie als besonders unangenehme Erscheinung eine ausgesprochene Mundtrockenheit mit borkiger Zunge. 7 Tage nach den Erstsymptomen begann zudem ein Verschwommensehen in der Nähe, welches sich innert 3 Tagen verstärkte, bis nur noch weit entfernte Konturen scharf gesehen wurden. Damals fühlte sich der Patient besonders müde und klagte über Kopfschmerzen sowie unsystematisierten, durch brüsken Übergang von liegender zu aufrechter Stellung provozierbaren Schwindel. Treppensteigen war wie Lastenheben gut möglich, jedoch sehr anstrengend. Die Arbeit musste nie unterbrochen werden, und der Patient unternahm sogar eine Bergwanderung. Dyspnoe hatte nie bestanden, allerdings kam es zu mehreren Episoden mit Hustenreiz. Inzwischen hatte sich auch eine Miktionsstörung mit Impotenz hinzugesellt.

Am 23. August, nach 28 Krankheitstagen, erfolgte die *erste neurologische Untersuchung*, welche bis auf die Feststellung einer Akkommodationslähmung bei normaler Lichtreaktion, jedoch tonischer Erweiterung der Pupillen normal ausfiel. Die *Allgemeinuntersuchung* ergab keine weiteren Gesichtspunkte, namentlich bestanden keine Hinweise mehr auf eine Blasenlähmung. Im gleichtags angesetzten *Tierversuch* mit der Maus konnte das Vorhandensein von Botulinustoxin im Patientenblut eindeutig verifiziert werden. Die *Akkommodationsbreite* war mit 3,5 Dioptrien nur noch mässig eingeschränkt. Der *Schirmer-Test* war mit 0,5 cm beidseits pathologisch. Die *Sialometrie* an der Parotis ergab mit 0,04 ml/min eine äusserst geringe Sekretionsmenge, die durch Stimulation mit Zitronensaft nur unwesentlich auf 0,11 ml/min erhöht werden konnte. Anlässlich einer kurzen Hospitalisation gewonnene Werte in Blut und Urin waren normal. Bei einem

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regelmässigen Puls von 80/min hielt sich der Blutdruck um 110/60 mm Hg.

Wir applizierten nun 50 ml polyvalentes *Botulismus-Serum* (Behringwerke) intraglutäal. Nach dieser Massnahme will der Patient eine leichte allgemeine Besserung bemerkt haben. Im Schirmer-Test fand sich eine Zunahme der Tränensekretionsmenge auf 1,2 cm beidseits. Die Sialometrie ergab nun noch etwas weniger Ruhespeichel. Ein weiterer Tierversuch fiel jetzt aber negativ aus.

37 Tage nach Krankheitsbeginn und 2 Tage nach der Serumtherapie gaben wir zusätzlich *Guanidinum hydrochloricum* (3mal 500 mg/24 Std.) per os. Das *Elektromyogramm* ergab keine signifikant pathologischen Werte (s. unten) und konnte somit nicht als Kriterium für einen allfälligen Therapieeffekt dienen. Am folgenden Tag habe der Patient zuhause nach einer warmen Dusche fast schlagartig wieder normal gesehen. Die Guanidinbehandlung wurde fortgesetzt und verursachte bei Einnahme jeder Tablette perioral sowie an den Händen Parästhesien. Nach einigen Tagen war die bisher hartnäckige, mit Klistieren und manueller Bauchpresse bekämpfte Obstipation in eine vorübergehende, jeweils von der Guanidineinnahme abhängige Diarrhöe übergegangen. Hier wurde die Medikation, die nach Meinung des Patienten die Wende herbeigeführt hatte, sistiert. Die Miktions- und etwas später auch die Potenzstörung hatten sich schon vor der Hospitalisation normalisiert. Die Mundtrockenheit mit zeitweiliger Dysphagie persistierte über annähernd 3 Monate, und auch nachher klagte der Patient über ein merkwürdiges Gefühl, wie wenn der Mund wieder trocken würde, obwohl dies nach der Speichelmenge zu schliessen nicht zutreffend gewesen sei.

Eingehende neurologische *Kontrolluntersuchungen* nach 5½ und 8 Monaten ergaben normale Resultate bis auf eine immer noch leicht pathologische Hypolakrimation im Schirmer-Test. Sämtliche Beschwerden waren jedoch verschwunden.

Der gesamte Krankheitsverlauf ist in Abb. 1 dargestellt.

Verlauf, Untersuchungsergebnisse und Therapie bei 9 Patienten

Latenz: Das bei 5 Patienten sicher bestimmbare Intervall zwischen Einnahme des gifthaltigen Käses und dem Auftreten erster Krankheitszeichen betrug im Mittel 37 Std., minimal 4, maximal 108 Std.

Erstsymptome: Ohne Regel konnten sowohl gastrointestinale Erscheinungen als auch Obstipation, Mundtrockenheit, Verschwommensehen oder Schwindel als erste Erkrankungszeichen auftreten. Die okulären Symptome traten meistens später auf als die gastrointestinalen. Die Patienten suchten den Arzt meist wegen der rasch einsetzenden, sehr beeindruckenden Sehstörung auf.

Symptomatologie (Abb. 2): Das Krankheitsbild war durch das regelmässige Vorkommen von Verschwommensehen und Mundtrockenheit mit Dysphagie markant charakterisiert. Zwei Drittel der Erkrankten waren obstipiert. Mehrmals, jedoch nicht obligat, wurden Nausea und Erbrechen sowie Durchfälle und kolikartige Bauchschmerzen im Anfangsstadium angegeben. Frappant waren Veränderungen der Psychodynamik mit Unlust, Abgeschlagenheit, Desinteresse und mangelndem Beharrungsvermögen, die wir unter dem Be-

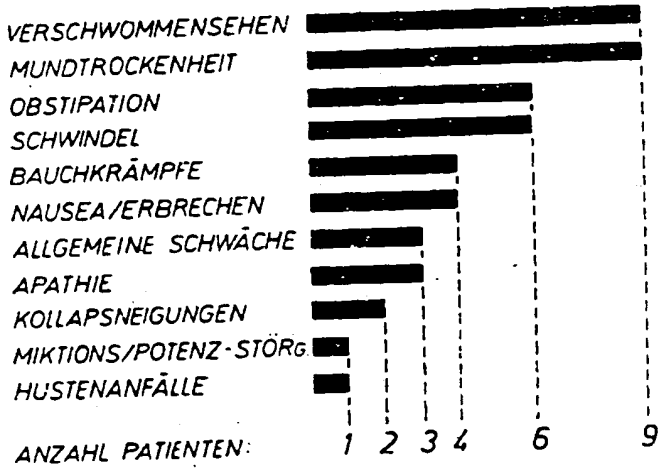

Abb. 2. Häufigkeit der Symptome bei 9 Patienten mit Botulinusintoxikation Typ B.

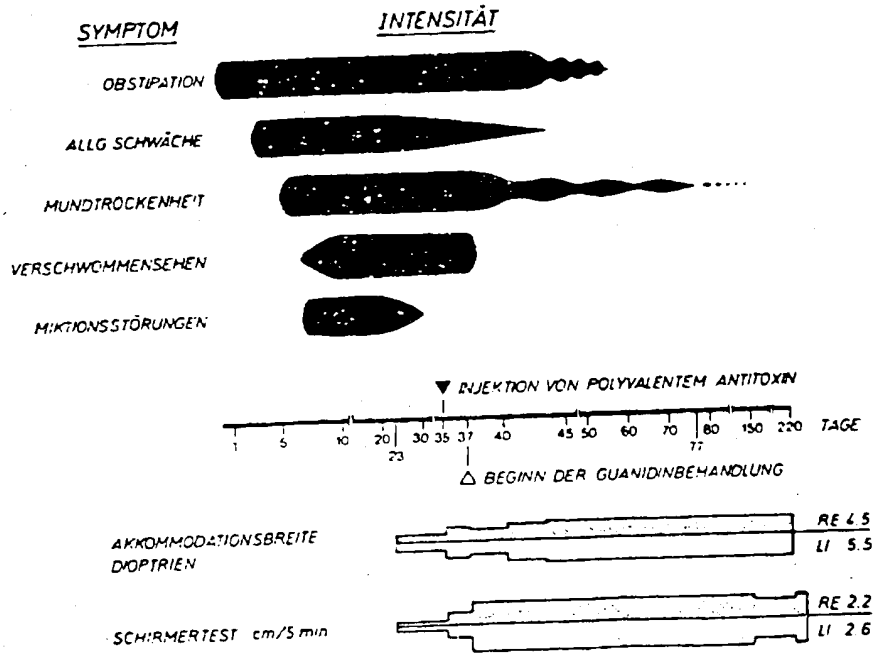

Abb. 1. Patient D. G. Synopsis des Verlaufs einer leichten Vergiftung mit Botulinustoxin Typ B.

rin Apathie subsumieren. Über eine eigentliche somatomotorische Schwäche berichteten ebenfalls einige Patienten, nicht jedoch über Erscheinungen, die man eindeutig als Muskelparesen bezeichnen könnte. Wie MONIER [52] würden wir die Schluckstörungen viel eher als blosse Folge der Speichelsekretionsstörung denn als Ausdruck einer bulbären Innervationsstörung interpretieren. Die Patienten konnten nämlich mühelos und ohne Regurgitation trinken oder angefeuchtete Bissen, nicht jedoch völlig trockene Speisen schlucken. Schwindel war ein häufiges Symptom, welches meist einem sogenannten unsystematisierten Schwindel entsprach, zweimal jedoch einer Kollapsneigung, wie wir sie von der orthostatischen Hypotonie kennen. In einem einzigen Fall bestanden Miktions- und Potenzstörungen. Ebenfalls alleinstehend war die Angabe über Anfälle eines episodischen, trockenen Hustens, möglicherweise in Zusammenhang mit einer gestörten Bronchosekretion.

Neurologische Untersuchung: Im Neurostatus fanden wir als einzige relevante Befunde einerseits regelmässig eine Abweichung des Nahpunktes aus der Lesedistanz infolge Akkommodationslähmung, anderseits 2mal eine verzögerte Lichtreaktion der Pupillen. Der Umstand, dass weder an Hirnnerven noch an Rumpf und Extremitäten Paresen nachweisbar waren, verdient besondere Beachtung.

Allgemeinuntersuchung: Dieselbe fiel wie Routineuntersuchungen in Blut und Urin regelmässig normal aus.

Tierversuch: (Mit Antitoxin geschützte Mäuse, denen toxinhaltiges Blut appliziert wurde, konnten überleben, wogegen ungeschützte Tiere unter den Zeichen eines Botulismus starben.) Ein solcher Versuch wurde bei allen Patienten durchgeführt und fiel 5mal positiv aus, wobei die Krankheitsdauer bis zum Test 7 bis 28 Tage betragen hatte. Bei den 4 negativen Resultaten erfolgten die Bestimmungen zwischen dem 18. und dem 28. Krankheitstag. Ein negatives Resultat dieses an sich hochspezifischen Versuchs schliesst das Vorliegen eines Botulismus keineswegs aus. Einerseits könnte eine zu geringe Menge noch vorhandenen freien Toxins hiefür verantwortlich sein, anderseits aber auch bereits eine völlige Elimination der Giftkörper. Bei der gesicherten Gemeinsamkeit der Intoxikationsquelle und den Übereinstimmungen im klinischen Bild bestand bei diesen Patienten ohnehin kein Zweifel mehr an der Diagnose.

Akkommodationsbreite: Die ophthalmologischen Bestimmungen ergaben im Anfangsstadium bei 2 Patienten 0, jedoch nie mehr als 3 Dioptrien. Fortgesetzte Kontrollen erlaubten eine Überwachung des Verlaufs bis zur Normalisierung.

Schirmer-Test (Abb. 3): (Nach Einlegen eines Filterpapierstreifens in den anästhesierten Konjunktivalsack wird die durch Tränenflüssigkeit innerhalb von 5 min befeuchtete Strecke gemessen. Normalerweise darf mit einer Strecke von 1.5–3 cm gerechnet werden, wobei [illegible] bei grosser individueller Schwankungsbreite und Unterschiedlichkeit im Vorgehen und verwendeten Material eine absolut quantitative Bedeutung abgesprochen werden muss [22]. Wir verwendeten Lackmuspapier.) Die befeuchtete Strecke betrug bei den Erstuntersuchungen durchschnittlich 9 mm, minimal 3 mm, maximal 16 mm/5 min. Dieser Umstand mag die Klagen einzelner Patienten über brennende Augen erklären, wenngleich auch das Verschwommensehen ebensolche Beschwerden implizieren könnte. Die Tests konnten mühelos wiederholt werden und zeigten im weiteren Verlauf mit einer Ausnahme (S. B.) eine Sekretionszunahme. An 4 Fällen beobachteten wir, dass bei bereits normalisierter Akkommodationsbreite der Schirmer-Test mit 9–11 mm/5 min noch immer geringe Sekretionsmengen ergab.

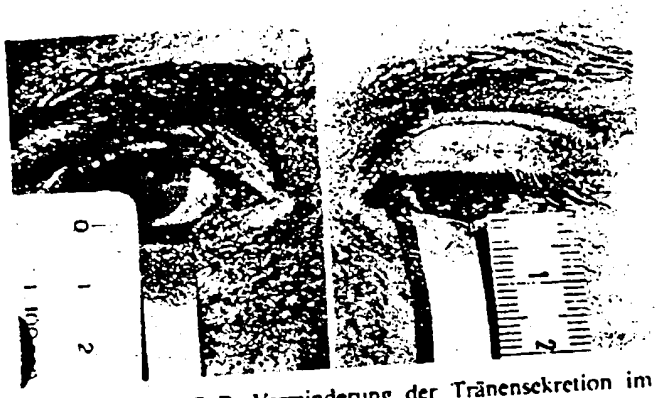

Abb. 3. Patient S. B. Verminderung der Tränensekretion im Schirmer-Test. – a) Rechtes Auge: 25 Tage nach Erkrankungsbeginn. – b) Linkes Auge: 7 Monate später.

Sialometrie: Otorhinolaryngologen leiteten den Parotisspeichel zur Messung der Sekretionsmenge über eine in den Ductus parotideus eingeführte Kanüle ab. Die so bei 4 Patienten gewonnenen Resultate betrugen für den Ruhespeichel durchweg weniger, als dies der mittleren Ruhesekretion beim Gesunden (0.03 ml/min) entsprechen würde. Durch Stimulation mit Zitronensaft – eine Untersuchung, die bei innervationsgestörter Sekretionsverminderung als spezifisch gilt – konnte bei 3 Fällen eine geringe, jedoch ungenügende Reizantwort provoziert werden [25, 55].

Elektroenzephalographie: In 2 Fällen wurden Hirnstromkurven abgeleitet, welche normal ausfielen.

Elektromyographie: (Der linke N. ulnaris wurde am Handgelenk mit Nadelelektroden supramaximal gereizt. Vor der Untersuchung wurde der Nerv mit Scandicain 2% oberhalb des Ellenbogens blockiert; dadurch konnte eine unerwünschte willkürliche Aktivierung des untersuchten Muskels vermieden werden, und die Untersuchung wurde weniger schmerzhaft. Mit Oberflächenelektroden wurden die Summenpotentiale vom M. adductor pollicis elektromyographisch abgeleitet. Gleichzeitig wurden die Kontraktionen dieses Muskels unter isometrischen Bedingungen registriert. Die Hauttemperatur über dem untersuchten Muskel wurde immer konstant auf 36–37° C gehalten.) Bei 4 Patienten wurden folgende Untersuchungen durchgeführt: repetitive Reizung mit 2/sec während 90 sec,

mit 10/sec während 4 sec und mit 20/sec während 1 min. Nach einer tetanischen Reizung mit 50/sec während 1 sec wurde mit Einzelreizen während 10 min nach einer posttetanischen Erschöpfnug oder Fazilitierung gesucht. Bei keinem der Patienten konnten Befunde erhoben werden, die signifikant von der Norm abwichen. Wiederholte Untersuchungen bei 3 der Patienten vor und nach Behandlung (Antitoxingabe und Guanidin) zeigten nie eine signifikante Veränderung der Befunde.

Therapie: 5 Patienten, davon 4 mit positivem Tierversuch, erhielten zwischen dem 10. und dem 35. Krankheitstag eine intraglutäale Injektion von 50 ml eines polyvalenten (A, B, E) *Botulinusantitoxins*. Ein in einem Fall nach 7 Tagen wiederholter Tierversuch ergab nun ein negatives Resultat, was auf eine Elimination vorher noch vorhandenen freien Toxins schliessen lassen könnte. Ob der Verlauf im übrigen günstig beeinflusst werden konnte, lässt sich aus dem vorliegenden Krankengut nicht schliessen (vgl. Abb. 4). 2 Patienten, beide mit positivem Tierversuch, erhielten ab 32. bzw. 35. Krankheitstag während 10 Tagen ausserdem *Guanidinum hydrochloricum* (3mal 500 mg/24 Std.) per os. Beide Patienten gaben kurz nach Behandlungsbeginn einen Rückgang der Sehstörung, ein Patient zudem eine auffällige Beeinflussung der Obstipation mit Übergang in Diarrhöe an. Als Nebeneffekte wurde in beiden Fällen über Nausea, Schwindel und heftige Kribbelparästhesien einmal an den Händen und einmal an den Füssen geklagt. Da die vorgängige elektromyographische Untersuchung keine relevanten Befunde ergeben hatte, war ein objektiver Nachweis der Guanidinwirkung nicht möglich.

Verlauf (Abb. 4): Innerhalb weniger Tage bildeten sich stets Erbrechen und akute gastrointestinale Erscheinungen zurück, gefolgt von einer Verminderung des Krankheitsgefühls mit allgemeiner Schwäche und unsystematisiertem Schwindel. Nach und nach normalisierte sich auch der Stuhlgang. Die letzten Beschwerden bezogen sich 6mal auf Mundtrockenheit und 3mal auf Verschwommensehen infolge Akkommodationslähmung. Die Symptome dauerten wenigstens 20 Tage lang, bei 2 Patienten länger als 70 Tage. Eine Patientin klagte auch nach dieser Zeit noch über Mundtrockenheit, wobei eine Sialometrie der rechten Parotis nach wie vor keine messbare Speichelsekretion ergab. Der Schirmer-Test bei 4 symptomfrei gewordenen Patienten fiel bemerkenswerterweise durchweg noch pathologisch aus. Zu beachten ist ferner die Feststellung bei einer Patientin, welche auch nach vielen Monaten über eine vorher nicht gekannte Empfindlichkeit mit Augenbrennen bei Windexposition klagte und bei welcher der Schirmer-Test nach 7 Monaten eine noch geringere Sekretionsmenge angab als während der eigentlichen Erkrankung (vgl. Abb. 3).

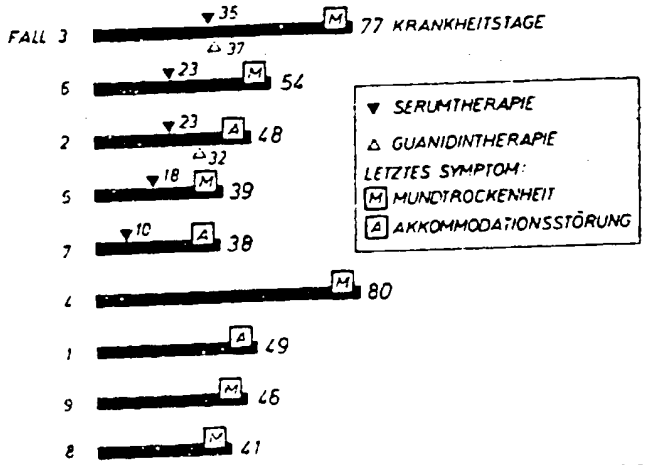

Abb. 4. Krankheitsdauer, Therapie und letztes Symptom bei 9 Patienten mit Botulismus Typ B.

Diskussion

Botulinustoxin

Das Toxin ist ein durch das Clostridium botulinum produziertes Protein, das in reiner Form eines der stärksten Gifte darstellt. Die Intoxikation erfolgt durch Ingestion verdorbener, oft konservierter Speisen, wie Fleisch, Fisch, Gemüse und anderes. Eine parenterale Vergiftung beim sogenannten Wundbotulismus ist äusserst selten [45]. Die serologisch differenten Typen A, B, C, D, E, F, von denen nur A, B und E – bei uns besonders B – von Bedeutung sind, unterscheiden sich auch nach Molekulargewicht und Aminosäurenkomposition [41]. Bei speziesabhängiger Empfindlichkeit gegenüber bestimmten Toxintypen (D-Toxin lähmt die Blase von Ratten wirksamer als A-Toxin [5]) sind die Vergiftungssymptome stets dieselben [39]. Ungleiche Auswirkungen kommen praktisch in der Letalität botulinusintoxizierter Menschen zum Ausdruck. Die Letalität dürfte zwar allgemein abgenommen haben [28], ist aber für A-Vergiftungen höher als für B-Vergiftungen [3, 18] und beträgt bei stark divergenten Angaben und unter Ausschluss extremster Werte 10 bis 65% [18, 31, 53, 64, 67].

Pathophysiologie

Die meisten Intoxikationserscheinungen erklären sich durch eine Störung der cholinergischen Übertragung, insbesondere am neuro-muskulären Übergang, aber auch an den prä- und postganglionären parasympathischen und einzelnen postganglionären sympathischen Nervenendigungen sowie möglicherweise am Nebennierenmark [26].

Nachdem der periphere Wirkungsort schon länger bekannt gewesen war [17, 21, 33] konnte Ambache [2] erstmals die selektive Wirkung des Toxins auf die cholinergische Übertragung demonstrieren. Die Theorie der quantenmässigen Freisetzung des Azetylcholins an der motorischen Endplatte [36] dient heute als Voraussetzung für das Verständnis des Botulinuseffektes auf die neuro-muskuläre Übertragung (Abb. 5). Das durch Nervenimpulse in den Synapsenspalt freigesetzte und durch Membrandepolarisation die Muskelfaser er-

Schweiz. med. Wschr. *104*, Nr. 19 (1974)

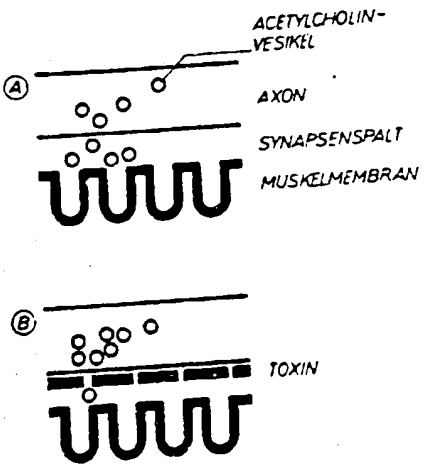

Abb. 5. Wahrscheinlicher Wirkungsmechanismus von Botulinustoxin an der motorischen Endplatte. A = normale Transmission, B = Interferenz mit Toxin.

regende Azetylcholin findet sein elektronenoptisches Korrelat wahrscheinlich in den präsynaptisch lokalisierten sogenannten Vesikeln. Nach mehrheitlicher Auffassung behindert das Botulinustoxin die Freisetzung dieser Azetylcholinquanten, ohne deren Synthese oder Speicherung zu stören [6]. An Ratten appliziertes, ferritinmarkiertes Toxin konnte im Synapsenspalt sichtbar gemacht werden [71]. Wahrscheinlich kommt es zu einer Fixation des Toxins, welches den Übertritt einer genügend grossen Anzahl von Vesikeln auf den postsynaptischen Bereich verhindert [24]. Hiermit vereinbar sind die elektromyographischen Erfahrungen, wonach repetitive Stimuli am motorischen Nerv im Sinne einer Fazilitierung grössere Muskelsummenpotentiale erzeugen als Einzelreize [9, 43, 58], aber auch die experimentelle Beobachtung einer Frequenz- und Amplitudenverminderung von Miniaturendplattenpotentialen am botulinusvergifteten Tier [66].

Eine toxinbedingte Synthesestörung [51], wie sie auch kombiniert mit einer Verlangsamung des Axoplasmastroms postuliert wurde [48, 49], wirkt unter dem Gesichtspunkt der Fazilitierung wenig überzeugend, müsste doch bei repetitiver Freisetzung von Azetylcholin eher mit einer Abnahme der depolarisierenden Wirkung infolge Erschöpfung der Reserven im präsynaptischen Bereich gerechnet werden.

Lichtoptische Untersuchungen neuraler und muskulärer Elemente eines an Botulismus Verstorbenen brachten keine strukturellen Veränderungen zur Darstellung [68]. Elektronenmikroskopisch konnte jedoch experimentell gezeigt werden, dass das Toxin zu einer Denervation mit histochemisch korrelierbaren Regenerationsvorgängen führt [19, 20]. Diese Beobachtungen konnten kürzlich durch Untersuchungen an Muskeln eines Botulinuskranken mit Nachweis von Strukturveränderungen im Bereich der Endplatte ergänzt werden [32], wobei eine Ähnlichkeit mit dem Eaton-Lambert-Syndrom [52] auffiel. Dieses myasthenische Syndrom weist auch neurohumorale Gemeinsamkeiten mit dem Botulismus auf [23]. Es sind dies die beiden einzigen bisher bekannten Affektionen, denen vermutlich eine präsynaptische Störung der Azetylcholinfreiger Quantenzahl zugrundeliegt.

Ein zentraler Angriffspunkt des Toxins wurde auch immer wieder diskutiert [56, 69]. Der Tod infolge Ateminsuffizienz wurde sowohl einem rein muskulären Mechanismus [47] als auch einer direkten Wirkung auf das Atemzentrum [1] zugeschrieben. Als Hinweis auf einen zentralen Angriffspunkt wurden EEG-Veränderungen mit Perioden elektrischer Stille bei intoxizierten Affen [56], aber auch Allgemeinveränderungen mit intermittierendem Abflachen der Kurve bei Botulinuskranken [16] geltend gemacht. Anderseits liegen auch Berichte über normale Hirnstromkurven vor [30, 38], denen wir unsere zwei Beobachtungen beifügen können. Obwohl Fälle mit zerebraler Beteiligung in Form von Bewusstseinstrübungen bekannt sind [18, 38], stellt dies die Ausnahme dar, und GRAEBNER und CLEELAND [30] konnten bei schwer Befallenen mit ausgesprochenen Paresen psychometrisch normale Hirnleistungen nachweisen.

Die Wirkungsweise des Toxins im autonomen Nervensystem bleibt vorderhand unbekannt.

Die dargelegten Verhältnisse sind in ihrer Relation zum klinischen Bild in Abb. 6 zusammengefasst.

Klinisches Bild

Die Diagnose Botulismus ruft in uns zunächst die Vorstellung eines schwersten Zustands mit fataler Prognose wach. Ein derartiges *klinisches Vollbild* entwickelt sich nach einer mehrstündigen bis mehrtägigen Latenzperiode und wird oft eingeleitet durch Nausea, Erbrechen und kolikartige Bauchschmerzen. Gleichzeitig oder etwas später machen sich eine allgemeine Müdigkeit und Schwäche bemerkbar. Das nun einsetzende Lähmungsbild wird häufig angeführt durch den Ausfall somatomotorischer Hirnnerven mit Ptose, Diplopie, Dysphagie und Dysphonie. Zuerst auf Arme oder Beine übergreifend schreiten die Lähmungen bis zur schlaffen Tetraplegie mit Ateminsuffizienz fort. Als Folge der autonomen Innervationsstörung kommt es zu einer internen Ophthalmoplegie mit Akkommodationslähmung und mydriatischer, areflektorischer Pupille sowie über denselben Mechanismus zu einer allgemeinen Hyposekretion, zu einem paralytischen Ileus mit Blasenatonie und einer Tachykardie. In schweren Fällen tritt unter dem Bild des Atemstillstands, gelegentlich auch des akuten Herzversagens, der Exitus ein.

In zahlreichen Publikationen wird allerdings über *mildere Krankheitsverläufe* berichtet [44, 46, 62]. In dieser Weise weichen auch unsere Fälle eindeutig vom eben geschilderten, als lebensbedrohlich zu betrachtenden Vergiftungsbild ab. Als leichteste und isolierte Zeichen können Mundtrockenheit [29, 40] oder Augensymptome [40, 57] vorliegen. In Mitteilungen über gesicherte B-Intoxikationen [35, 38, 52, 58] finden sich Angaben über verschiedene Schweregrade. Der Fall einer 40jährigen Patientin von JOUGLARD u. Mitarb. [35] mit wahrscheinlicher B-Intoxikation und fast ausschliesslich autonomen Störungen ist mit unseren Pa-

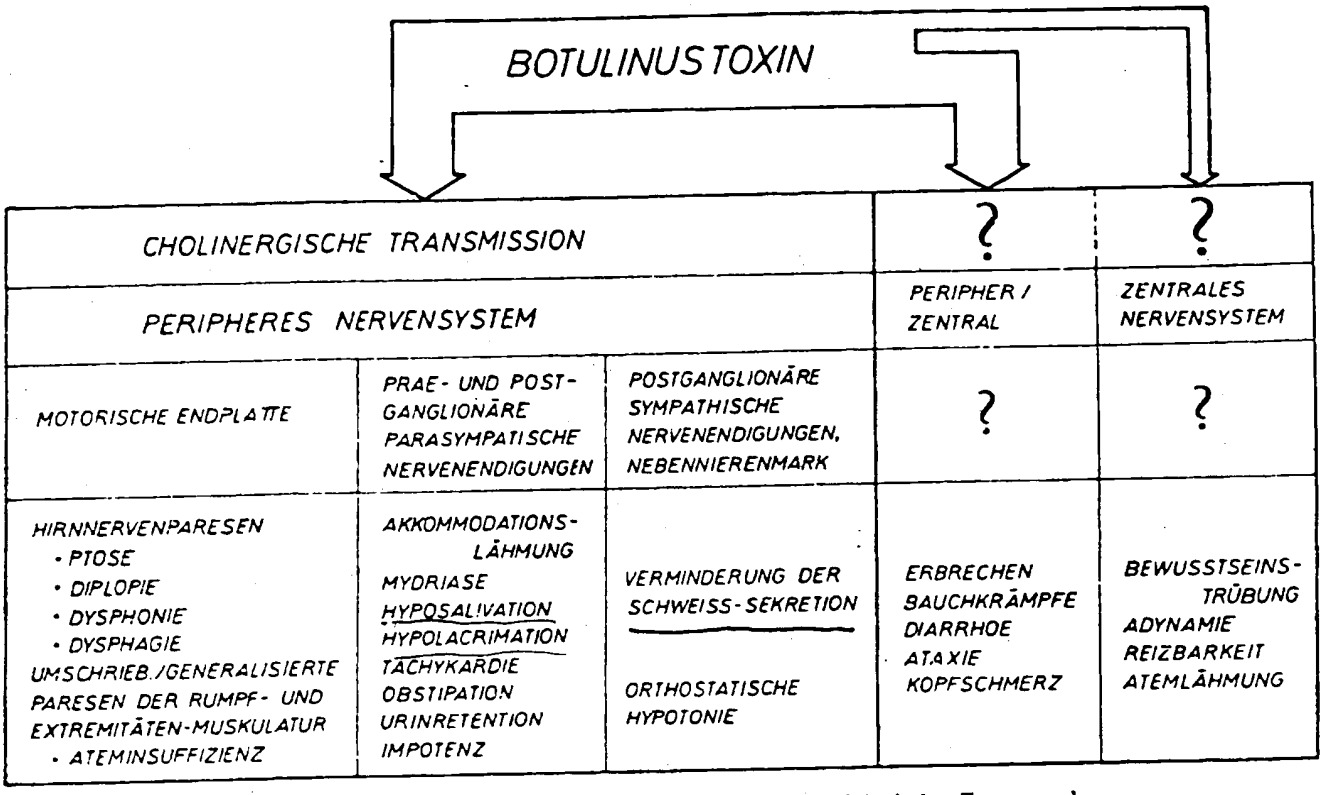

Abb. 6. Symptome der Botulinusintoxikation und deren wahrscheinlicher pathophysiologischer Zusammenhang.

tienten gut vergleichbar. Wie bei diesen erfolgte die Erstuntersuchung einige Wochen nach Krankheitsbeginn. Wir zogen zwar einen bereits remittierten neuromuskulären Befall in Erwägung, konnten aber anamnestisch keine stichhaltigen Argumente finden. Etwa eine Ptose oder ein paralytischer Strabismus – welcher in diesem Zusammenhang leicht zu verwechseln ist mit «Doppeltsehen» infolge dioptrischer Visusstörungen – hätten unseren durchweg differenzierten Patienten auffallen müssen. An verschiedenen Beispielen schwerer A-Vergiftungen muss beachtet werden, dass autonome Funktionen nicht zwangsläufig betroffen werden [7, 9, 27, 61]. Wir möchten demzufolge anhand unserer Patienten auf die Möglichkeit einer speziellen Affinität des B-Toxins zur autonomen Innervation hinweisen.

Entgegen den Erwartungen bei einer leichten Vergiftung haben die Symptome überraschend lange persistiert. Die Akkommodationsstörung als offensichtliches Krankheitszeichen wurde oft durch die weniger auffällige Mundtrockenheit überdauert. Mit dem Schirmer-Test und der Sialometrie konnte unseres Wissens erstmals gezeigt werden, dass durch die Botulinusintoxikation eine das übrige Krankheitsbild bis Monate überdauernde Sekretionsstörung an Tränendrüse und Parotis entstehen kann. In Analogie zu den Beobachtungen von DUCHEN [20] an der motorischen Endplatte könnte es sich dabei um die Folgen einer toxinbedingten Denervation handeln, welche durch Zeit beanspruchende Regenerationsvorgänge kompensiert werden muss.

Therapie

Die Verdachtsdiagnose Botulismus genügt in der Regel, um eine sofortige Therapie mit polyvalentem *Antitoxin* einzuleiten. Entscheidend ist die *Aufrechterhaltung von Atmung und Kreislauf*, notfalls mit reanimatorischen Massnahmen. Mittlerweile kann das Resultat des hochgradig spezifischen Mäuseversuchs abgewartet werden.

Über die Frage der Berechtigung zur nicht risikolosen Serumtherapie besteht allerdings keine Einigkeit. Verschiedenen Autoren befürworten ein solches Vorgehen generell [4, 35, 38, 52]. MINERVIN [50] empfiehlt sogar eine zusätzliche perorale Serumgabe in der keineswegs allgemein anerkannten Auffassung, dass das Toxin protrahiert aus dem Magen-Darm-Trakt resorbiert werde. Demgegenüber unterstreichen IIDA u. Mitarb. [34] und ONO u. Mitarb. [54] die Bedeutung einer möglichst frühzeitigen Gabe, da das Toxin vor Erreichen des Wirkungsortes und definitiver Bindung neutralisiert werden sollte. Die volle Ausprägung der Symptomatik wird, wie wir dies auch bei unseren Patienten verfolgen konnten, nach einigen Tagen erreicht. Selbst wenn im Tierversuch die Präsenz wirksamer Toxinbestandteile am längsten bis am 28. Krankheitstag nachgewiesen werden konnte, war damit die Pathogenität derselben für die Kranken nicht zwangsläufig belegt. Einen sicheren therapeutischen Effekt durch das stets spät verabreichte Antitoxin konnten wir in keinem Fall beobachten, ebenso auch kein eindeutig abweichendes Verhalten der Unbehandelten. Nachdem wir

nun die gesamte Evolution dieser Erkrankungen überblicken können, würden wir diese Massnahme heute nicht mehr für angezeigt halten. Vielmehr empfehlen wir auf Grund dieser Erfahrung, bei bereits langdauernden leichten Vergiftungen mit regredienter Symptomatik selbst bei positivem Tierversuch auf die risikobehaftete Antitoxinbehandlung zu verzichten.

In diesem Sinne möchten wir die Notwendigkeit eines gegenüber den schweren Vergiftungsbildern differenzierenden und damit adäquaten therapeutischen Verhaltens unterstreichen. *Guanidin*, welches früher bei Myasthenie verwendet wurde und in letzter Zeit zur Behandlung des Eaton-Lambert-Syndroms empfohlen wird [23], muss als Adjuvans [10] in der Behandlung des Botulismus berücksichtigt werden. Nachdem Masland und Gammon [43] elektromyographisch keine günstige Beeinflussung des Toxineffektes angeben konnten, beschrieben Cherington und Ryan [8] erstmals eine Zunahme der Summenpotentiale am Thenar eines Vergifteten bei supramaximaler Reizung des N. medianus. Dieser Effekt wurde einer vermehrten Azetylcholinfreisetzung zugeschrieben [63]. Sowohl beim A-Botulismus [27, 60] als auch beim B-Botulismus [11] wurde die Nützlichkeit von Guanidin aber auch in Frage gestellt. Ricker und Doell [58, 59] wiederum konnten sowohl tierexperimentell als auch am Menschen elektromyographisch einen positiven Effekt bei B-Intoxikation demonstrieren: Unter Guanidin erfolgte eine Abnahme der Fazilitierung bei repetitiver Stimulation. Die beiden Autoren [59] haben zudem eine klinische Besserung, erstmals auch autonomer Funktionen, beschrieben. Günstige Resultate erzielten Cherington und Greenberg ausserdem mit Germin allein [12] oder in Kombination mit Guanidin [13, 15], wogegen sich Echothiophat als wirkungslos erwies [14]. Während dem Guanidin ein präsynaptischer Wirkungsort zukomme, sei derjenige des Germins postsynaptisch [13].

Bei unseren 2 Fällen darf in Analogie zu den Beobachtungen von Ricker und Doell [59] eine günstige Wirkung auf die autonomen Funktionsstörungen angenommen werden. Die Nebeneffekte, von denen wir besonders die akralen Parästhesien erwähnen möchten, wurden von den Patienten ohne weiteres in Kauf genommen und bildeten sich unmittelbar nach Absetzen des Medikaments vollständig zurück. Insgesamt scheint es uns vertretbar, die Verabreichung von Guanidin in der durch Ryan und Cherington [61] angegebenen Dosierung von 15–35 mg/kg/24 Std. zu empfehlen.

Differentialdiagnose

Besonders unter Einbezug der leichten Vergiftungsfälle ist die Differentialdiagnose der Affektionen mit verwechselbarem Erscheinungsbild breit und reicht über andere Vergiftungen und neurologische Erkrankungen bis in den ausschliesslich internistischen Bereich. Tabelle 1 sind aus der Literatur gesammelte Angaben [8, 11, 16, 18, 31, 35, 44, 45, 51, 58, 70] zusammengestellt.

Tabelle 1
Differentialdiagnostisch in Betracht zu ziehende Affektionen

Vergiftungen	Neurologische Affektionen	Sonstige
Atropin inkl. Belladonnapräparate	zerebrovaskulärer Insult	akutes Abdomen
Aminoglykosid-Antibiotika	Diphtherie	Myokardinfarkt
Bariumkarbonat	Eaton-Lambert-Syndrom	Salmonellosen
CO	Enzephalitis	
Diphenylhydantoin	Guillain-Barré-Syndrom	
Methylalkohol	Hirntumor	
Methylchlorid	Lues cerebrospinalis	
Muscheln	multiple Sklerose	
organische Phosphorverbindungen	Myasthenia gravis	
Pilze	myatrophische Lateralsklerose	
Vipergift	Poliomyelitis	

Die Schwierigkeit der Diagnose Botulismus liegt bei schweren wie leichten Fällen in der teilweisen Imitation verschiedenster Zustände sowie in der ausgesprochenen Seltenheit, welche die zutreffende Assoziation nicht unmittelbar aufkommen lässt. Einer unserer Patienten erhielt vom Hausarzt wegen Bauchkoliken ein atropinähnliches Spasmolytikum, welches zunächst nicht unberechtigt für die Augenstörung und die Mundtrockenheit verantwortlich gemacht wurde. Die Mehrzahl der Befallenen suchte wegen der beeindruckenden Sehstörung zuerst den Augenarzt auf. Sämtliche Patienten gehörten einer Altersklasse an, bei welcher noch keine wesentliche Presbyopie vorhanden sein konnte. Wir rechnen deshalb mit einer gewissen Zahl nicht erkannter bzw. verkannter Botulismusfälle bei älteren Personen, die über dieses Leitsymptom nicht verfügen konnten.

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Résultats thérapeutiques de la synoviorthèse à l'acide osmique

J. Boussina, I. Kuzmanovic, W. Esselinckx et G. H. Fallet

Résumé. Une synoviorthèse à l'acide osmique a été pratiquée sur 59 genoux chez 31 malades qui présentaient tous une synovite chronique avec épanchement récidivant, rebelle au traitement général et local. L'injection comportait 100 mg d'acide osmique, conjointement avec 5 ml de Xylocaïne à 2% et 80 mg de Codelcortone-TBA.

Les effets de la synoviorthèse ont été contrôlés à intervalles très réguliers, selon divers critères cliniques, objectifs et subjectifs. Les effets cliniques ont été chiffrés en pourcentage d'amélioration par rapport à l'état initial. Ces chiffres individuels ont permis d'obtenir des moyennes pour l'ensemble des malades, en fonction des délais d'observation, à la suite de la synoviorthèse.

Mis à part une poussée inflammatoire passagère dans les heures qui suivent la synoviorthèse, on assiste à une amélioration objective et subjective. Durant les quatre premiers mois, l'amélioration objective se situe autour de 60% et l'amélioration subjective autour de 80%. Par la suite on assiste, autour du 7e mois, à un fléchissement du pourcentage d'amélioration correspondant à la période la plus fréquente de récidive de la synovite. Au-delà du 8e mois on constate une tendance à une amélioration progressive et continue et, au 32e mois, durée maximum d'observation de notre série, l'amélioration peut être chiffrée en moyenne à 90% objectivement et 95% subjectivement.

Pour l'ensemble de nos cas (59 synoviorthèses), nous avons observé 22 rechutes, soit 37%. Ces résultats sont comparables, dans l'ensemble, à ceux de la synovectomie chirurgicale, mais la simplicité relative de la synoviorthèse à l'acide osmique plaide en faveur de l'application précoce de ce traitement dans les cas de synovite chronique rhumatismale.

Summary. Osmic acid synovectomy was performed on 59 knees in 31 patients all suffering from chronic synovitis with recurrent effusions resistant to systemic and local treatment. The injection consisted of 100 mg of osmic acid, 5 ml of 2% Xylocain and 80 mg of Codelcortone-TBA.

The effects of the synovectomy were checked at regular intervals using various objective and subjective clinical criteria. The clinical effects were recorded as percentage improvement of the original state and these individual figures were used to calculate the average for the group of patients at given intervals after synovectomy.

Apart from some temporary inflammation in the few hours following the synovectomy, a subjective and objective improvement was noted. During the first 4 months the objective improvement was about 60% and subjective improvement about 80%. Later, at about the 7th month, there was a fall in the percentage of improvement corresponding to the period where recurrence of the synovitis was most frequent. After the 8th month, a tendency towards progressive improvement was noted. This persisted, and at the 32nd month, length of maximum follow-up in our series, the recorded improvement was an average of 90% objective and 95% subjective improvement.

In our group of cases (59 synovectomies) 22 relapses (37%) occurred. These results are comparable with those for surgical synovectomy, but the relative simpli-

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Type B Botulism

Report on mild courses with predominantly autonomic nervous impairment

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Abstract. On the basis of 9 cases, a mild course of a type B botulinus intoxication with autonomous function disturbances yet no demonstrable neuromuscular involvement is presented. After describing methods for objective observation of the course of the illness, basic information is given on pathophysiological relationships, as well as remarks on treatment and differential diagnosis. Observations reveal an unexpectedly long persistence of tear duct and parotid gland functional disorders, usually no longer subjectively perceptible.

Present experience counsels against an antitoxin treatment in the late phase of a mild botulism undergoing remission. On the other hand, the use of guanidine can be considered relatively [adjective omitted].

In correlation with literature data, the possibility of a special affinity of the B-toxin for the human autonomic innervation is pointed out.

Summary. 9 cases of human type B botulism with mild course and almost selective effect upon autonomic nervous functions are described. After clinical remission a reduction of lacrimal and salivary secretions could be demonstrated for up to several months. Our experience argues against the use of antitoxin in the late course of such cases with minor symptomatology but in favour of guanidine, which offered some subjective improvement. From comparable published cases it seems that autonomic nervous functions in the human are particularly susceptible to B type toxin.

During the course of a small epidemic in Switzerland in the summer of 1973, due to French Brie, at least 48 persons were poisoned by botulinus toxin type B. Thorough investigations enabled an exact reconstruction of the epidemiological conditions [37]. Nine of these victims, 5 women and 4 men with an average age of 31 years, were investigated at our clinic, including a brief hospitalization in order to perform more far-reaching investigations.

Description of the case. The case of a 42 year old businessman is typical and shall be presented in detail:

D. G. on 25 July 1973 ate around 100 g of the incriminated cheese, whose taste was supposedly not affected. Twelve hours later on, there was a feeling of bloating and constipation. After 2-3 days, the patient was unusually listless, could not work efficiently, and was pensive. Meanwhile, a general weakness had developed, as well as an especially unpleasant pronounced dryness of the mouth with crusted tongue. Furthermore, 7 days after the first symptoms, close-range vision became blurred, intensifying over the course of 3 days, until only very distant contours appeared sharp. At that time, the patient felt

especially tired and complained of headache and general dizziness, provoked by rapid change from a lying to an erect posture. He was quite able to climb stairs and lift loads, but with great effort. He never had to interrupt his work, and the patient was even able to go on a hike. There was never any dyspnoe, but there were several episodes of tickling in the throat. Difficulty during urination and impotence had also developed in the meantime.

On 23 August, after being sick for 28 days, the *first neurological investigation* was performed, which turned out to be normal, except for finding an accommodation paralysis with normal photoreaction, but tonic dilation of the pupils. The *general examination* revealed nothing further, specifically, no further indication of cystoparalysis. *Animal testing* with a mouse, carried out on the same day, clearly verified the presence of botulinus toxin in the patient's blood. The *range of accommodation* at 3.5 diopters was only moderately impaired. The *Schirmer test* at 0.5 cm on either side was pathological. *Sialometry* of the parotid gland revealed an extremely low quantity of secretion at 0.04 ml/min, which stimulation with lemon juice was only able to increase slightly to 0.11 ml/min. Blood and urine tests during a brief hospitalization were normal. The blood pressure was 110/60 mm Hg with a regular pulse of 80 bpm.

We now applied 50 ml of polyvalent *Botulism serum* (Behringwerke) intragluteally. After this procedure, the patient will have noticed a slight general improvement. In the Schirmer test, there was an increase in tear duct secretion to 1.2 cm on either side. The sialometry now still revealed somewhat low baseline saliva. But an additional animal experiment now turned out negative.

37 days after the commencement of the illness and 2 days after the serum therapy, we additionally administered *Guanidinum hydrochloricum* (3x 500 mg/24 h) per os. The *electromyogram* revealed no significantly pathological values (see below) and thus could not serve as a criterion for a general therapeutic effect. On the following day, after taking a hot shower at home, the patient recovered normal vision almost instantly. The guanidine treatment was continued and upon taking each tablet paresthesias were produced perioral and at the hands. After several days, the so far stubborn constipation that had been treated with enemas and ventral pressure changed into a transient diarrhea which was dependent on the guanidine dose. At this time, the medication (which the patient felt had brought about the change) was halted. The difficulty during urination and, somewhat later on, also the impotence had already normalized prior to the hospitalization. The dryness of the mouth with occasional dysphagia persisted for approximately 3 months, and even afterwards the patient complained of a peculiar feeling, as if his mouth were again dry, even though this was not so, judging from the quantity of saliva.

Extensive neurological *checkups* after 5 ½ and 8 months revealed normal results, except for a remaining slightly pathological hypolacrimation in the Schirmer test. However, all symptoms had disappeared.

The overall course of the disease is shown in figure 1.

Course, Findings, and Therapy for 9 Patients

Latency: The definitely determined interval between ingestion of the toxic cheese and the occurrence of the first symptoms in 5 patients was 37 hours on average, the minimum being 4 and the maximum 108 hours.

Initial symptoms: Both gastrointestinal symptoms as well as constipation, dryness of the mouth, blurred vision or vertigo can occur as the first signs of the illness. The ocular symptoms usually occurred later than the gastrointestinal ones. The patients for the most part consulted a physician as a result of the rapid onset of very noticeable disturbed vision.

Symptomatology (Figure 2): The clinical picture was distinctly characterized by the regular occurrence of blurred vision and dryness of the mouth with dysphagia. Two-thirds of the victims were constipated. Often, though not necessarily, nausea and vomiting, as well as diarrhea and colic-like abdominal pain were reported in the initial stage. There were striking changes in the psychodynamics with unpleasure, fatigue, disinterest and lack of drive, which we subsumed under the term apathy. Some patients also reported a kind of somatomotor weakness, but nothing that could be clearly termed muscular paresis. Like MONIER [52], we interpret the disturbed swallowing as a mere consequence of the disturbed secretion of saliva, rather than a manifestation of disturbance of the bulbar innervation. The patients were able to drink effortlessly and without regurgitation, or swallow moistened bits of food, but not completely dry food. Vertigo was a frequent symptom, usually corresponding to a generalized vertigo, but on two occasions there was a tendency to collapse, as is familiar during orthostatic hypotonia. In a single patient there was difficulty with urination and impotence. Likewise, there was a single report of an episodic, dry coughing, possibly in connection with a disturbed bronchosecretion.

Neurological investigation: The only relevant findings in the neurostatus was a deviation of the near point from the reading distance as a result of accommodation paralysis, and on two occasions a delayed photoreaction of the pupils. The fact that there was no demonstrable paresis, either at the cranial nerves or at the trunk and limbs, deserves special attention.

General examination: Generally normal in blood and urine, as during routine examinations.

Animal experiment: (Mice protected with antitoxin, to whom toxin-containing blood was administered, survived, whereas unprotected animals died from botulism). Such an experiment was carried out for all patients and the outcome was positive on five occasions, the duration of the illness up to the time of the test having been 7 to 28 days. In the case of the 4 negative outcomes, the determinations were done between day 18 and 28 of the illness. A negative result for this highly specific test in no way rules out the occurrence of botulism. On the one hand, the quantity of free toxin still present might be too low; on the other hand, the toxins might have already been fully eliminated. But given the certain commonality of the intoxication source and the correlations in the clinical pattern of these patients, there was no longer any doubt as to the diagnosis.

Accommodation range: The ophthalmological determinations revealed zero diopters in 2 patients during the initial stage, but never more than 3 diopters. Continued checkups enabled a monitoring of the course until normalization.

Schirmer Test (Figure 3): (After inserting a strip of filter paper in the anesthetized conjunctival sac, the length moistened by lacrimal fluid within 5 minutes is measured. Normally, one expects a length of 1.5-3 cm, yet because of great individual fluctuations and differences in technique and material used one cannot say that the method is absolutely quantitative [22]. We used litmus paper.) The moistened length during the first investigations was on average 9 mm, the minimum being 3 mm and the maximum 16 mm/5 min. This fact may explain why several patients complained of burning eyes, although the blurred vision could also produce such symptoms. The tests were easily repeated afterwards and revealed an increased secretion in the further course, with one exception (S. B.). In 4 cases, we observed that the quantity of secretion remained low at 9-11 mm/5 min, even though the accommodation range of the Schirmer test had already normalized.

Sialometry: Ear, nose and throat specialists inserted a cannula into the Ductus parotideus and drained the parotid saliva to measure the quantity of secretion. The results obtained in this way from 4 patients were consistently less for the baseline saliva than the average baseline secretion of a healthy person (0.03 ml/min). Stimulation with lemon juice - a test considered specific for reduced secretion with disturbed innervation - was able to provoke a slight, but inadequate stimulus response in 3 cases [25, 55].

Electroencephalography: Brain currents were plotted in two cases and proved to be normal.

Electromyography: (The left N. ulnaris was stimulated supramaximally with needle electrodes on the wrist. Prior to the test, the nerve was blocked with 2% scandicaine above the elbow; this prevented an unwanted voluntary activation of the investigated muscle and made the test less painful. Using surface electrodes, the cumulative potentials of M. adductor pollicis were tapped electromyographically. At the same time, the contractions of this muscle were registered under isometric conditions. The skin temperature above the investigated muscle was always kept constant at 36-37°C.) The following investigations were carried out in 4 patients: repetitive stimulation with 2/sec for 90 sec, with 10/sec for 4 sec and with 20/sec for 1 minute. After a tetanic stimulation with 50/sec for 1 sec, we looked for a posttetanic exhaustion or facilitation with single stimuli for 10 minutes. No findings significantly different from normal were found in any of the patients. Repeated tests in 3 of the patients before and after treatment (antitoxin and guanidine) revealed no significant change in the findings at any time.

Therapy: Five patients, including 4 with positive animal experiment, received an intragluteal injection of 50 ml of a polyvalent (A, B, E) *Botulinus antitoxin* between day 10 and 35 of the illness. In one case, an animal experiment repeated after 7 days now showed a negative result, which suggested an elimination of previously still evident free toxin. As to whether the course was favorably influenced in other respects cannot be concluded from the given population (cf. Figure 4). Two patients, both with positive animal experiment, also received *Guanidinum hydrochloricum* (3x 500 mg/24 h) per os for 10 days, starting on day 32 and 35 of the illness. Shortly after the beginning of treatment, both patients reported a decrease in the visual disturbance, and one patient also reported a significant influencing of the constipation with a change to diarrhea. In both

cases, side effects reported were nausea, vertigo, and strong tingling sensations, once on the hands and once on the feet. Since the previously electromyographic investigation had revealed no relevant findings, an objective verification of the guanidine effect was not possible.

Course (Figure 4): Within a few days, the vomiting and acute gastrointestinal symptoms always receded, followed by a lessening of the sick feeling with general weakness and generalized vertigo. Defecation also gradually normalized. The last symptoms were dryness of the mouth on six occasions and blurred vision due to accommodation paralysis on three occasions. The symptoms lasted for at least 20 days, and in two patients longer than 70 days. Even after this time, one patient still complained of dryness of the mouth, and sialometry of the right parotid gland still revealed no measurable secretion of saliva. The Schirmer test in 4 patients who had become asymptomatic was strangely enough still pathological. It should also be mentioned that one patient complained of sensitivity and burning of the eyes when exposed to wind even many months thereafter, and her Schirmer test after 7 months indicated even less secretion than during the actual illness (cf. Figure 3).

Discussion

Botulinus toxin

The toxin is a protein produced by Clostridium botulinum, which in pure form is one of the strongest of poisons. Intoxication occurs by ingestion of spoiled, often preserved foodstuffs, such as meat, fish, vegetables, and others. A parenteral poisoning in so-called wound botulism is extremely rare [45]. The serologically different types A, B, C, D, E, F, of which only A, B and E - especially B in our country - are significant, also differ in terms of molecular weight and amino acid composition [41]. The intoxication symptoms are always the same [39], even though the sensitivity to certain toxin types is food-dependent (D-toxin more effectively paralyzes the rat bladder than does A-toxin [5]). In practice, different effects are manifested by the mortality of botulinus-intoxicated humans. Although mortality has generally diminished [28], it is higher for A-intoxications than for B-intoxications [3, 18] and, even though the data are highly divergent and when the most extreme values are removed, it amounts to 10 to 65% [18, 31, 53, 64, 67].

Pathophysiology

Most of the intoxication symptoms are explained by a disturbance of the cholinergic transmission, especially at the neuromuscular junction, but also at the pregangliar and postgangliar parasympathetic and certain postgangliar sympathetic nerve endings, and possibly also at the adrenal medulla [26].

While the peripheral site of the effect had been known rather long [17, 21, 33], AMBACHE [2] was the first to demonstrate the selective action of the toxin on the cholinergic transmission. The theory of the quantum release of acetylcholine at the motor end plate [36] today serves as a prerequisite for understanding the botulinus effect on the neuromuscular transmission (Figure 5). The acetylcholine released into the synaptic cleft by nerve impulses and stimulating the muscle fibers by membrane depolarization has its electron-optical counterpart probably in the presynaptically localized so-called vesicles. Most believe that the botulinus toxin hinders the release of these acetylcholine quanta, without disturbing their synthesis or buildup [6]. Ferritin-labeled toxin administered to rats was identified in the synaptic cleft [71]. There is probably a fixation of the toxin, which prevents a sufficiently large number of vesicles from crossing to the postsynaptic region [24]. This is consistent with electromyographical findings, wherein repetitive stimuli at the motor nerve generate larger cumulative muscle potentials by a facilitation than do single stimuli [9, 43, 58]; it also explains the experimental observation of a decrease in frequency and amplitude of miniature end plate potentials in the botulinus-intoxicated animal [66].

A toxin-dependent disturbance of synthesis [51], as has been postulated also in combination with a slowdown of the axoplasm current [48, 49], is less convincing from the standpoint of facilitation, and under a repetitive release of acetylcholine there should instead be a decrease in the depolarizing effect due to depletion of reserves in the presynaptic region.

Light microscope studies of neural and muscular elements of a person who died from botulism revealed no structural alterations [68]. However, electron microscope studies revealed that the toxin had caused a denervation with histochemically correlated regeneration processes [19, 20]. Recently, these observations have been amplified by studies on the muscles of a botulinus patient with demonstration of structural alterations in the region of the end plate [32], similar to the Eaton-Lambert Syndrome [52]. This myasthenic syndrome also reveals neurohumoral similarities to botulism [23]. Thus far, these are the only two ailments known whose presumptive cause is a presynaptic disturbance of the release of acetylcholine with deficient number of quanta.

There has also been continuing discussion of a central point of attack of the toxin [56, 69]. Death by respiratory insufficiency has been attributed to both a purely muscular mechanism [47] and a direct action on the respiratory center [1]. As proof of a central point of attack, EEG alterations with periods of electrical quiet in intoxicated monkeys [56], as well as general alterations with intermittent flattening of the curve in botulinus patients [16] have been cited. On the other hand, there are also reports of normal brain currents [30, 38], to which we can add our two observations. Although there are known cases with cerebral involvement in the form of disorientation [18, 38], these are the exception, and GRAEBNER and CLEELAND [30] were able to demonstrate psychometrically normal brain performance in severe cases with pronounced paresis.

For now, the method of action of the toxin in the autonomic nervous system remains unknown.

The presented relationships are summarized in figure 6 in relation to the clinical pattern.

Clinical pattern

The diagnosis of botulism initially invokes the notion of a severe condition with fatal prognosis. Such a *full clinical picture* develops after a period of latency of many hours or many days and is often ushered in by nausea, vomiting and colic-like abdominal pain. At the same time or somewhat afterwards a general fatigue and weakness become evident. The paralysis which now sets in is often preceded by failure of somatomotor cranial nerves with ptosis, diplopia, dysphagia and dysphonia. The paralysis, at first of the arms or legs, gradually extends to the point of flaccid tetraplegia with respiratory insufficiency. As a result of the disturbance of autonomic innervation, there is an internal ophthalmoplegia with accommodation paralysis and mydriatic, areflective pupils and, through the same mechanism, a general hyposecretion, a paralytic ileus with bladder atonia, and a tachycardia. In severe cases, death occurs with respiratory failure, occasionally also acute heart failure.

In numerous publications, however, *mild courses* have been reported [44, 46, 62]. In this way, our cases also differ clearly from the just-described, life-threatening intoxication. Dryness of the mouth [29, 40] or symptoms of the eyes [40, 57] may occur as the mildest and isolated symptoms. Various degrees of severity are reported in papers on verified B-intoxications [35, 38, 52, 58]. The case of a 40 year old patient of JOUGLARD et al. [35] with probable B-intoxication and almost exclusively autonomic disturbances is quite comparable to our patient. As with her, the first investigation occurred several weeks after the start of the illness. Although we considered a neuromuscular attack already in remission, we could find no valid proof of this in the

prior medical history. A ptosis or a paralytic strabismus - which in this regard is easily confused with "double vision" due to dioptric visual disturbances - would have made our thoroughly differentiated patient stand out. It must be noted from examples of severe A-intoxications that autonomic functions are not necessarily affected [7, 9, 27, 61]. Consequently, we would like to point out, with our patient, the possibility of a special affinity of the B-toxin for the autonomic innervation.

Contrary to expectations for a mild intoxication, the symptoms persisted surprisingly long. The accommodation disturbance, which is the obvious symptom of the ailment, was often outlasted by the less noticeable dryness of the mouth. With the Schirmer test and the sialometry we were able to show, for the first time to our knowledge, that the botulinus intoxication can produce a disturbed secretion of the tear ducts and parotid gland that lasts for months beyond the other clinical picture. By analogy with the observations of DUCHEN [20] on the motor end plate, this might be the result of a toxin-related denervation, which has to be compensated by time-consuming regeneration processes.

Therapy

The suspected diagnosis of botulism is generally enough to institute an immediate therapy with polyvalent *antitoxin*. The critical goal is to *maintain respiration and circulation*, if necessary with resuscitation. In the meantime, one waits for the result of the highly specific mouse experiment.

However, there is no agreement as to whether the not risk-free serum therapy is justified. Various authors generally advise such a procedure [4, 35, 38, 52]. MINERVIN [50] even recommends an additional peroral administering of serum in the by no means generally acknowledged opinion that the toxin will be protractedly resorbed from the gastrointestinal tract. Accordingly, IIDA et al. [34] and ONO et al. [54] underscore the importance of the earliest possible administering, since the toxin should be neutralized before reaching the site of its action and definitive binding. The full manifestation of the symptomatology, as we were also able to track in our patients, is reached after several days. Even though it was possible to verify the presence of active toxin components at most until day 28 of the illness in the animal experiment, this did not necessarily prove its pathogenicity to the patient. We were not able to observe in a single case a certain therapeutic effect from the antitoxin, always given late, nor any clearly different behavior of untreated patients. Now that we have had a chance to survey the entire evolution of these illnesses, we no longer consider this procedure as indicated. Instead, on the basis of this experience, we recommend against the risky antitoxin treatment in mild intoxications of already long duration with receding symptomatology, even when the animal experiment is positive.

In this regard, we would like to underscore the need for an adequate therapeutic procedure which is differentiated from the severe intoxication cases. *Guanidine*, which has previously been used for myasthenia and has recently been recommended for treatment of Eaton-Lambert Syndrome [23], must be considered as an aid [10] in the treatment of botulism. While MASLAND and GAMMON [43] were not able to show any favorable influencing of the toxin effect with electromyography, CHERINGTON and RYAN [8] have described for the first time an increase in the cumulative potentials on the thenar

of an intoxication patient during supramaximal stimulation of the N. medianus. This effect has been attributed to an increased release of acetylcholine [63]. Yet the usefulness of guanidine has been questioned in both A-botulism [27, 60] and B-botulism [11]. RICKER and DOELL [58, 59], in turn, were able to demonstrate a positive effect during B-intoxication, both through animal experiments and human electromyography: under guanidine, there was a decrease in the facilitation during repetitive stimulation. Furthermore, the two authors [59] have described a clinical improvement, including autonomic functions for the first time. Moreover, CHERINGTON and GREENBERG achieved favorable results with germine alone [12] or in combination with guanidine [13, 15], whereas echothiophate proved ineffective [14]. While guanidine is ascribed a presynaptic site for its effect, that of germine is postsynaptic [13].

In our two cases, by analogy with the observations of RICKER and DOELL [59], a favorable action on the disturbances of autonomic function can be presumed. The side effects, of which we should mention in particular the acral paresthesias, were easily tolerated by the patients and completely receded immediately after stopping the medication. On the whole, we feel it is possible to recommend the administering of guanidine in the dosage scheme of 15-35 mg/kg/24 h, as indicated by RYAN and CHERINGTON [61].

Differential diagnosis

Especially when the mild courses of intoxication are included, the differential diagnosis of the symptoms with confusing pattern is broad and extends across other intoxications and neurological illnesses, reaching even into the exclusively internal medicine realm. Table 1 summarizes the information collected from the literature [8, 11, 16, 18, 31, 35, 44, 45, 51, 58, 70].

The difficulty of the botulism diagnosis in both severe and mild cases lies in the partial imitation of the most diverse conditions, as well as the considerable rarity, which does not immediately suggest the proper association. One of our patients was given an atropine-containing spasmolytic by his physician, which was initially believed to be responsible (not unwarranted) for the vision disturbance and the dryness of the mouth. Most of the patients first consulted an eye doctor because of the noticeable disturbed vision. All of the patients belonged to an age cohort in which no significant presbyopia should be present. We therefore expect a certain number of unrecognized or misdiagnosed botulism cases in older persons where this major symptom cannot be present.

We owe a special debt of thanks to the following colleagues for their collaboration: P. BLOK, E. CABERNARD, H. P. GRAF, H. GYSIN, R. HALDIMANN, P. HUBER, D. JACHERTZ, L. KUTAK, J. MOSIMANN, R. NYFFENEGGER, A. PETERMANN, J. VELVART. We thank Mr. P. SCHNEIDER for the drawings.

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ILLUSTRATIONS

Figure 1. Patient D. G. Synopsis of the course of a mild intoxication with botulinus toxin type B.

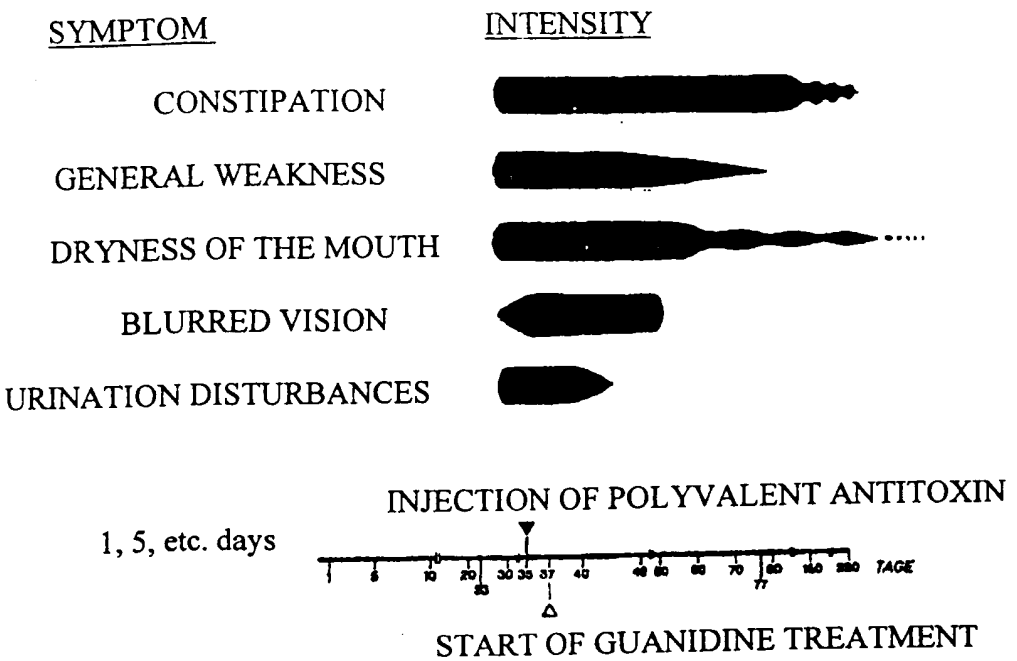


Figure 2. Frequency of symptoms in 9 patients with botulinus intoxication type B.

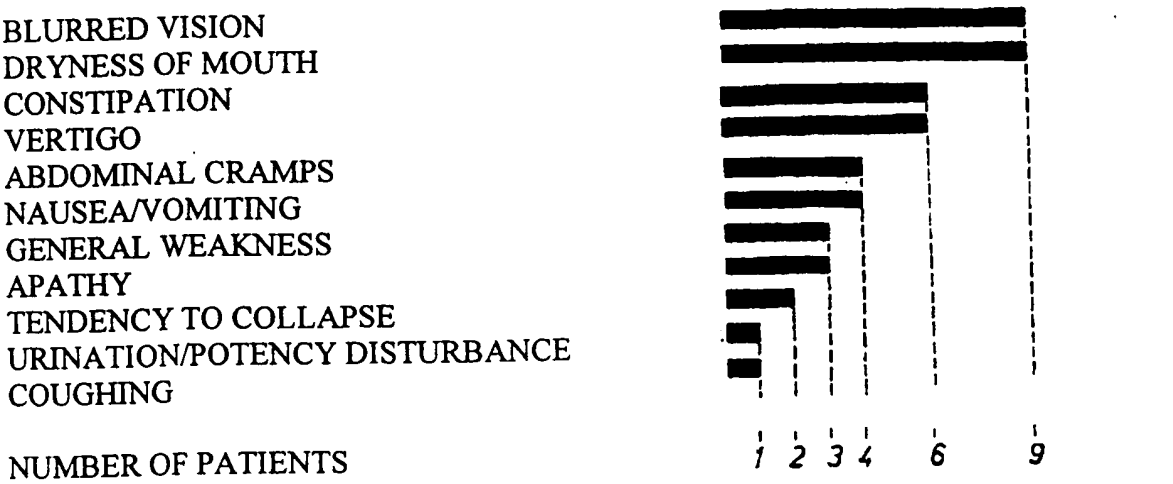


Figure 3. Patient S. B. Decrease in tear secretion in the Schirmer test. - a) Right eye: 25 days after start of illness. - b) Left eye: 7 months afterwards.

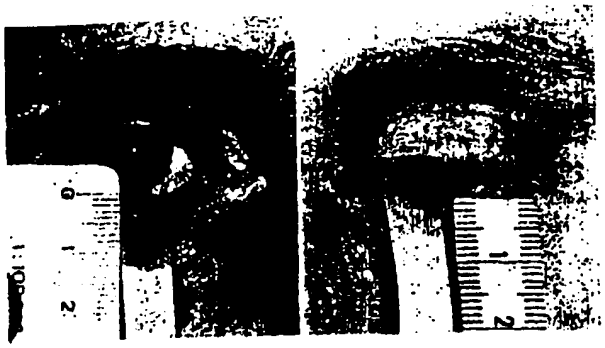
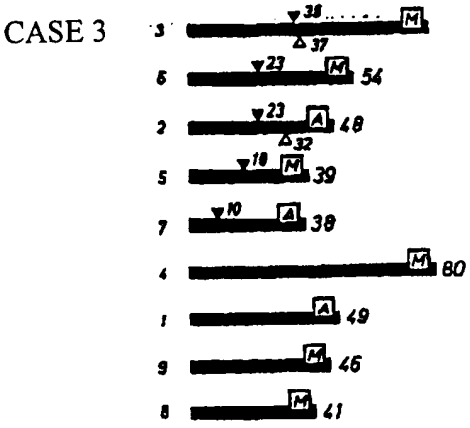


Figure 4. Duration of illness, therapy, and last symptom in 9 patients with botulism type B.



[legend]

▼ -SERUM THERAPY

△ -GUANIDINE THERAPY

LAST SYMPTOM:

M - DRYNESS OF MOUTH

A - ACCOMMODATION DISTURBANCE

Figure 5. Probable mechanism of action of botulinus toxin on the motor end plate. A = normal transmission, B = interference with toxin.

key:

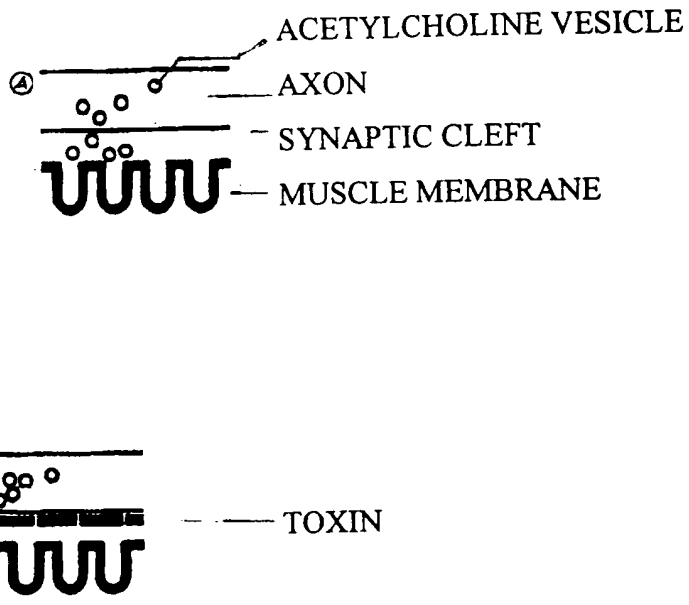


Figure 6. Symptoms of botulinus intoxication and their probably pathophysiological connection.

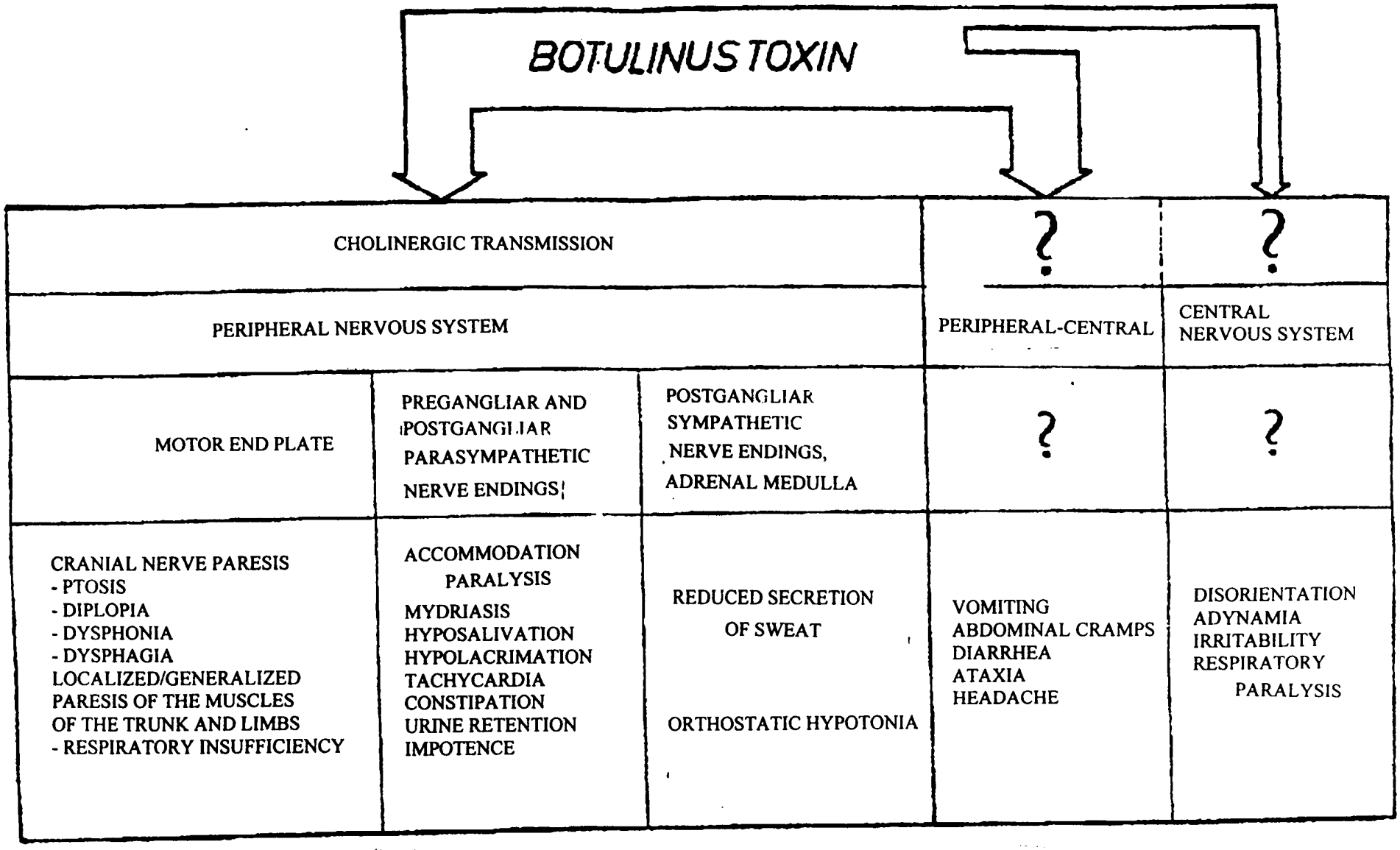


Table 1.
Symptoms for differential diagnosis

Intoxications	Neurological ailments	Other
Atropine incl. belladonna	stroke	acute abdomen
Aminoglycoside antibiotics	diphtheria	Myocardial infarct
Barium carbonate	Eaton-Lambert Syndrome	Salmonellosis
CO	Encephalitis	
Diphenylhydantoin	Guillain-Barré Syndrome	
Methyl alcohol	Brain tumor	
Methyl chloride	Lues cerebrospinalis	
Mussels	Multiple sclerosis	
Organic phosphorus compounds	Myasthenia gravis	
Mushrooms	myatrophic lateral sclerosis	
Viper venom	Poliomyelitis	

Clinical Sciences

Expedited Publication

Hemifacial Spasm Treated With Botulinum A Toxin Injection

Peter J. Savino, MD; Robert C. Sergott, MD; Thomas M. Bosley, MD; Norman J. Schatz, MD

• Fifteen patients with hemifacial spasm were treated with botulinum A toxin injections. All patients experienced relief from spasm, with the effect lasting an average of 12.2 weeks. Complications were tearing in four patients, inability to close the involved eye in three patients, corneal exposure in one patient, and ectropion in one patient. All complications were transient and deemed minor by the patients.

(*Arch Ophthalmol* 1985;103:1305-1306)

Hemifacial spasm (HFS) is a periodic contraction of the musculature of one side of the face. It occurs in adults, mostly women, and may be accompanied by mild ipsilateral facial weakness.[1] The contractions often begin as a twitching of the eyelids and gradually worsen to include episodic orbicularis muscle spasm and contracture of the ipsilateral facial musculature. Herein, we report the first prospective study of the role of botulinum A toxin injections for the treatment of HFS symptoms.

MATERIAL AND METHODS

All patients with HFS underwent a complete clinical neuro-ophthalmic evaluation. Computed tomographic scans were performed in all cases, but no mass lesions were identified.

Seventeen consecutive patients with HFS were injected with a diluted purified derivative of botulinum A toxin (Oculinum). This agent causes paralysis by producing a reversible neuromuscular blockade. One man died of a myocardial infarction two months after the injection, and another was unavailable for follow-up. Therefore, 12 women and three men who were injected were monitored. Some patients had multiple injections, so that there are 20 injections on which there are adequate follow-up data. Five injections were performed too recently to be included in our analysis.

The average age of the 15 patients was 63 years (range, 40 to 81 years). Four patients had right HFS, while in 11, the left side of the face was involved. The average duration of the HFS was 13.1 years (range, 0.5 to 35 years).

Nine patients had undergone previous treatments, including therapy with carbamazepine, haloperidol, diazepam, phenytoin, clonazepam, and lorazepam; biofeedback; and psychiatric therapy, without benefit. Hemifacial spasm recurred in two patients after they had undergone surgical microvascular decompression.

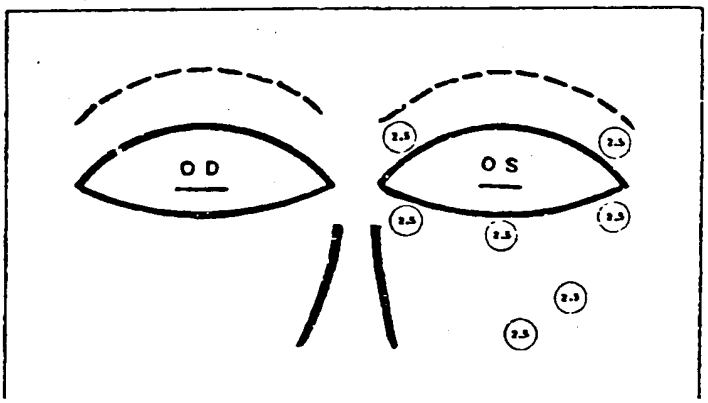
All patients were told of the investigative nature of the treatment and gave informed consent. One of us (P.J.S.) injected 2.5 units (0.001 μg/0.1 mL) (one unit is equal to the amount of toxin needed to kill 50% of a group of 18- to 20-g female Swiss-Webster mice [LD 50]) of botulinum A toxin into five periocular sites and two sites on the cheek (Figure). Patients were told that no attempt would be made to eliminate all of the spasm in the lower portion of the face, because this would result in a dense facial palsy. The optimum result of the injection was no orbicularis muscle spasm, little or no midfacial twitching, and a small degree of spasm at the angle of the mouth. All patients were told that some degree of facial weakness was anticipated.

RESULTS

All patients experienced relief from HFS following the injection(s) (Table). The mean time to improvement was 30.5 hours after injection (range, 12 to 48 hours). The mean duration of the effect was 12.2 weeks (range, four to 16 weeks). Only patient 4 had to undergo reinjection less than 12 weeks later because of a decrease in the effects of treatment.

Complications included tearing in four patients, complaints that the involved lid did not close in three

Sites of injection of botulinum A toxin (Oculinum) in treatment of hemifacial spasm.



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Summary of Data for Patients Injected With Botulinum A Toxin for Hemifacial Spasm*

Patient/ Age, yr/ Sex	Duration of Symptoms Prior to Injection, yr	Duration of Treatment Effects, wk	Complications
1/50/F	14	12	None
2/75/F	30	14	Eye not closing
3/66/F	14	12†	Eye not closing
4/75/M	10	14	None
5/56/M	15	12	None
6/69/F	8	8†	Tearing
7/81/F	0.5	12†	None
8/69/F	1.5	12	Tearing
9/60/F	17	12	Eye not closing
10/67/M	22	8	Tearing
11/54/F	20	16	None
12/44/F	3.0	12	None
		12	None
		12	None
13/65/F	7	16	Tearing; ectropion
14/74/F	35	16	Exposure
15/40/F	0.6	4	None
		12	None
		12	None
		16	None

*Note that patient 12 received three injections, and patient 15 received four injections.

†Time at last follow-up visit, with continued relief of hemifacial spasm.

patients, corneal exposure in one patient, and ectropion in one patient. Several complications were noted in some patients, so that most did not experience these side effects, all of which were temporary and disappeared within a maximum of two weeks. All patients had some facial weakness, but it was not severe or symptomatically troublesome in any patient. Questioning revealed that no patient was inhibited functionally by this facial weakness or the other complications. All patients considered their HFS to be greatly improved.

COMMENT

Hemifacial spasm is a disorder characterized by frequent twitching of the orbicularis oculi muscle, which then progresses to the twitching and then prolonged contraction of one side of the face. It is now generally accepted that idiopathic HFS results from compression of the facial nerve where it exits the brain stem, usually by an arterial loop of the posterior inferior cerebellar artery.[2] Although mass lesions may produce HFS by compressing the facial nerve in the posterior fossa, most patients do not have identifiable masses. Recent evidence has suggested that ectopic excitation and ephaptic transmission are important factors in the production of HFS.[3]

The symptoms of HFS are bothersome to patients to the point that some are unable to sleep or interact socially for employment or leisure. Rarely, but as occurred in two of our cases, the patient has only one functioning eye, which is visually useless during episodes of HFS.

A variety of treatment regimens have been suggested for HFS. Carbamazepine has been reported to alleviate HFS in approximately 50% of patients.[4] Several surgical procedures have also been reported to relieve HFS, but posterior fossa microvascular decompression, as described by Jannetta and coworkers,[2] appears to be the most effective. Jannetta[5] reported complete relief from HFS in 93% (213/229) of patients. Only five of the 229 patients had persistant HFS.[5] Complete relief was also reported in 97% (72/74) of patients operated on by Iwakuma and colleagues[6] when they employed this same surgical procedure.

A complication of posterior fossa microvascular decompression included hearing loss in 12 of 74 patients in one series[6] and in 18 of 229 patients in another.[5] Other complications encountered in this latter group of 229 patients included serous otitis media in eight patients, aseptic meningitis in 13 patients, and cerebrospinal fluid rhinorrhea in eight patients.

Botulinum A toxin, when injected into the orbicularis muscle, is effective in the treatment of essential blepharospasm or Meigs' syndrome.[7] We injected botulinum A toxin as shown in the Figure to determine its efficacy in treating the symptoms of HFS. All patients experienced prompt relief from symptoms. Moreover, no patient experienced systemic complications. There were eight instances of local side effects in 20 injections (Table). Despite these minor complications and the temporary nature of the relief of symptoms, all patients were extremely satisfied with the results of treatment. No patient refused reinjection when the HFS returned. Reinjection seemed to yield the same length of control as did initial injection.

Botulinum A toxin injection is a valid alternative in the treatment of HFS for patients who are unable to tolerate daily medication or unwilling to undergo neurosurgery.

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Effectiveness of botulinum toxin in the treatment of spasmodic torticollis

Boghen D; Flanders M

Eur Neurol 1993 June;33:199-203

Nineteen female and 15 males between the ages of 28-79 years with spasmodic torticollis were assessed quantitatively for posture deformity, tremor and range of neck movement, and qualitatively for pain and global subjective disability. All patients were then treated with botulinum toxin type A (supplied by Dr. Alan Scott) injections to appropriate neck muscles. Fifty-three treatments were administered using dosages of toxin in the range of 50-100 U per muscle. The maximum dose administered at a single setting was 280 U. The progress of the patients was assessed during an 18-month period. The authors report that 75% of patients showed documented improvement in both sujective and objective parameters and were considered treatment successes, and that pain improved in 65%, posture in 65%, tremor in 50% and range in 46%. The side effects that occurred were transient and included fatigue, dysphagia, neck weakness, hoarseness and local pain. The authors conclude that this study demonstrates that treatment with botulinum toxin is of significant benefit for the majority of patients with spasmodic torticollis.

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TORTICOLLIS
BOTULINUM TOXIN A
ADMINISTRATION AND DOSAGE
MUSCLES
EFFICACY
SAFETY
SIDE EFFECTS

03

Deadly toxin calms excited muscles

Nancy Heneson, Washington DC

BOTULINUM toxin is possibly the most poisonous substance known. But it has a kinder, gentler side: in tiny doses, it can relieve several serious disorders of movement. Last month, a panel of experts, convened by the National Institutes of Health (NIH) in Bethesda, Maryland*, recommended that the toxin be used more widely for a class of disorders known as the dystonias. In these conditions, involuntary muscle contractions cause twisting; repetitive, sometimes painful, movements; or abnormal postures.

Botulinum toxin, or "botox" as scientists know it, is usually associated with food poisoning, not medical treatment. But, ironically, the same qualities that make botox highly poisonous and sometimes fatal also make it an effective therapy.

Ten years ago, an American ophthalmologist in San Francisco hit on the idea that the muscle paralysis induced by the toxin might be put to clinical use in cases where patients suffered from excessive muscular activity. Alan Scott of the Smith-Kettlewell Eye Research Institute in San Francisco thought of using the toxin for strabismus, or misalignment of the eyes. In this condition, a person's eyes do not look in the same direction, and they are cross-eyed.

In the past decade, scientists have accumulated enough evidence to persuade the US Food and Drug Administration to approve injection of botox for strabismus, blepharospasm (forcible closure of the eyelids) and hemifacial spasm (muscle contractions on one side of the face). The NIH panel recommends that these uses be expanded to include other dystonias, which involve the neck, jaw, limbs, and vocal cords, as well as stuttering and rare, but quite troublesome, spastic closure of the anal or urinary sphincter. At present, the toxin is available from two sources: Smith-Kettlewell and the Centre for Applied Microbiological Research, Porton Down.

Botulinum toxin is a composite of two toxins—"binary" toxin and neurotoxin—together with an auxiliary protein, which acts as a stabiliser. When botulinum neurotoxin is ingested in spoiled food, the neurotoxin travels to the junctions of skeletal muscles and nerves, where it eventually blocks the release of the neurotransmitter acetylcholine, causing muscle weakness and, sometimes, paralysis.

According to Lance Simpson at the Jefferson Medical College in Philadelphia, Pennsylvania, no one knows exactly how the toxin reaches the acetylcholine receptor. However, researchers know that the neurotoxin consists of two linked chains of polypeptides, and that one chain binds to the muscle cell's plasma membrane while the other blocks the acetylcholine receptor.

If the chains are separated, says Simpson, toxicity is lost. This could be important for future manipulation of the toxin, says Simpson. For example, it may be possible to decouple the chains and attach a different kind of toxin or other drug to the binding chain, or change the binding site of the receptor-blocking chain.

For the time being, however, clinicians and researchers are happy with the neurotoxin as it is. Virtually everyone who has used the toxin in clinical trials reported their results at the NIH meeting, and the consensus was that when properly administered, the toxin is safe, effective, and, in some cases, the treatment of choice over surgery and drug therapy—particularly for focal dystonias, which affect only one or a few muscles in the body.

The response to localised injections of botox is far more reliable than that of other drugs such as anticholinergics and other neurotransmitter inhibitors. If a person is going to respond to botox, he usually does at the beginning of treatment, and continues to respond through repeated injections. Furthermore, botox is less drastic, less disfiguring, and less expensive than surgery (hundreds of dollars per botox treatment).

Of course, botox is not entirely problem-free. The panel stressed that the toxin is not a cure—nerves that the toxin blocks eventually sprout new terminals—so the blocking must be maintained through repeated injections. Although given in much smaller amounts than would be needed to produce botulism, it can still cause side effects, such as difficulty in swallowing, which result from excessive weakening of the target and surrounding muscles. However, the panel stated that such effects are relatively uncommon, usually transient, and often avoidable if clinicians are properly trained and muscle activity is accurately measured before treatment.

Furthermore, there is so far no report of any patient or handler of the toxin developing botulism or of the toxin adversely affecting patients with compromised immune systems. Indeed, the main problem with patients' response is that some form antibodies to the toxin. Scientists speculate that such a reaction could be the result of too much toxin in too short a period, or perhaps a genetic predisposition to immunity. □

*NIH Consensus Development Conference on Clinical Use of *Botulinum* Toxin, 12-14 November, 1990, Bethesda, Maryland.

Can monkeys read each others' minds?

WE OFTEN try to imagine another person's state of mind, so that we can modify our behaviour in the light of it. For instance, we often consider what other people's moods are likely to be, the desires they might have, and we guess the things that they might or might not know. But do other primates have this ability?

Dorothy Cheney and Robert Seyfarth of the University of Pennsylvania attempted to test this in the laboratory, with two species of macaque (*Animal Behaviour*, vol 40, p 742). There is some evidence that chimpanzees are able to attribute "mental states" to each other, but for all other primates, including the macaques, the evidence up until now is anecdotal.

Cheney and Seyfarth placed females in cages from which they could see a test arena. They put down a plate of appetising food, or sent in a threatening human "predator", who subsequently hid behind a screen.

In half of these trials, the researchers allowed a female's offspring to stay with her so that they could also see what was happening in the arena. But in the rest of the tests, the offspring saw nothing of what went on.

Cheney and Seyfarth then released the young of all these mothers into the arena. Half of the infants had prior knowledge of what they would find, and half of them had no idea.

If the females could attribute mental states to their young, reasoned the zoologists, then the mothers of the "ignorant" young ought to make more of a show of alerting their infants, for example, either to the food, with soft, hooting "food calls", or to the hidden "predator". But Cheney and Seyfarth found no difference in the behaviour of any of the mothers.

Perhaps the mothers did, indeed, recognise a difference between ignorant and knowledgeable young, but they just did not act on it. However, Cheney and Seyfarth believe it is more likely that macaques cannot put themselves in another's place, unlike humans. They seem unable to understand that individuals can differ from themselves, and from other monkeys, in the beliefs that they have. **Georgia Mason**

Ardea

Macaque mothers: fail to warn offspring of danger

Therapy with Botulinum Toxin

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6

Botulinum Toxin Type B: Experimental and Clinical Experience

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INTRODUCTION

Botulinum toxins BTXs (types A, B, C1, D, E, F, and G) are among the most potent toxins known. The toxins have been studied since the turn of the century, initially to gain an understanding of botulism, a form of food poisoning. Later, they were studied as something of a curiosity, because of the uniquely long-lasting and specific muscle paralysis induced by minute amounts of the toxins. Today, that "curiosity" is beginning to be exploited in the treatment of movement disorders such as blepharospasm and torticollis.

The type B toxin is possibly the first BTX ever discovered, having been identified as the causative agent in a 1895 outbreak of botulism (1). In this incident, which took place in Ellezelles, Belgium, three musicians died. The cause of death appeared to be a neuroparalytic toxin produced by an anaerobic, spore-forming bacterium. When these bacteria were cultured, the culture medium was found to cause botulism-like toxicity in a variety of experimental animals, by various routes of administration. It was later found that this toxicity was not prevented by protective antisera produced against toxin from bacteria isolated from a different incident of botulism, in Germany. Similarly, antisera protective against the Belgian toxin were not protective against the German toxin, although the bacteria that yielded the two toxins, and the toxicity they produced, were similar (2). Although both cultures were later lost, it is believed that the second type of *Clostridium botulinum* was a strain of what we today call type A (3). Thus began a long list of publications related to the bacteria and their toxins: there are far more publications related to BTXs in the following years than there have been documented cases of human botulism.

STRUCTURE OF BOTULINUM TOXIN TYPE B

Toxin–Nontoxic Protein Complex

As isolated from the bacterial culture medium, type B toxin (like type A toxin) is found combined with nontoxin proteins. In the case of type B toxin, stable complexes of two different sizes are formed. These complexes have sedimentation coefficients of 16 and 12 S, and are called L (large) and M (medium), respectively. (The uncomplexed, pure toxin protein is sometimes called the S, or small, form.) The M complex contains nontoxin proteins that reportedly do not have hemagglutinin activity (4). The larger of the two types of complex (L form, about 450–500 kD) contains other protein(s), in addition to these nontoxin proteins, that do have hemagglutinin activity. These toxin–nontoxin protein complexes are not held together by covalent bonds and can reversibly dissociate, releasing the toxin protein, depending on the pH and ionic strength of the solution. Both pH greater than 8 and low ionic strength favor dissociation of the complex. There is some antigenic cross-reactivity and sequence homology between the hemagglutinins of the type A and B toxins (5). However, neutralization of type B hemagglutinin activity with a type A antitoxin prepared against a type A toxin–hemagglutinin complex does not neutralize the type B toxin (6). Formation of an association complex with the nontoxin proteins appears to stabilize the activity of BTXs, perhaps by helping to maintain a necessary secondary or tertiary structure (7). It is presumably for this reason that the only currently commercially available BTX for clinical use, type A, is formulated in the form of a toxin–hemagglutinin-containing nontoxin protein complex, rather than as a formulation of the pure toxin.

Toxin

The type B toxin is synthesized by the bacteria as a single protein chain that has low activity until proteolytically cleaved. This cleavage—"nicking"—occurs endogenously by the action of bacterially produced protease(s) (8,9). Strains that activate most of the produced toxin are termed "proteolytic," such as the strains "Beans" and "Okra." Full activation of the toxin can also be achieved artificially by trypsinization: the bacterial nicking protease and trypsin cleave at or very near the same site. The amount of endogenous activation varies according to the *C. botulinum* type B strain and the fermentation conditions. Even under the best conditions, using the proteolytic strains, both nicked and unnicked toxin may be produced (10).

The nicked toxin has a molecular weight of about 150,000 kD and is composed of a heavy and light chain, held together by a disulfide bond and noncovalent bonding. Reduction of the disulfide bond causes a separation of the chains and loss of toxicity (11): neither chain by itself is toxic. The molecular weights of the two chains are about 100,000 and 50,000 kD, respectively (12). The nicking site is one-third of the length of the single chain, and the light chain is formed from the amino-terminal portion. The amino-terminal sequences of the unnicked type B are identical to the amino-terminus of the light chain of the nicked toxin (13). Whether this proteolytic cleavage termed "nicking" is sufficient to activate the toxin remains controversial (14).

Until very recently, only limited structural information about the type B toxin was available. Most of this information was derived by inference from antigenicity studies (although there was a limited amount of sequence data obtained from fragments of the toxin). The antigenicity data suggested significant differences between the various types

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of BTX in terms of their amino acid sequences. The antigenic differences are so significant and reproducible, in fact, that the botulinum bacteria are primarily classified by the antigenic specificity of the toxins they produce, with additional subdivision by group and by strain. Assignment to group is based on slight differences in culture characteristics (proteolytic ability, heat resistance of spores, optimal temperature for growth). Various type B bacterial strains within these groups have been described, primarily on the basis of the first source of culture. Some amino acid sequence differences between type B toxin produced by different strains of type B *C. botulinum* (15,16) have been reported, and even, in some cases (depending on the strain), antigenic differences (17). However, neutralizing type B antitoxin raised against one strain of type B bacterial toxin is protective against type B toxin produced from a variety of strains of type B bacteria (18).

The structure of the type B toxin is loosely related to that of tetanus toxin, also produced by bacteria of the genus *Clostridium*. The general size and subunit structure of the two classes of toxin are similar, and some aspects of mechanism of action also appear to be comparable. Antipeptide antibody binding studies indicate some limited sequence homology exists between various BTXs and tetanus toxin, particularly in specific regions of the purified (possibly partially unfolded) proteins (19). However, human and mouse polyclonal antibodies that neutralize tetanus toxicity do not cross-react with the BTXs. This implies that the homologous sequences in the primary structure of tetanus and botulinum toxins are generally short amino acid segments that are either not accessible or not immunogenic in the native conformation state of the molecules. The functional implications of the sequence homology are not yet understood but may be of great interest in understanding the mechanism of action of the various toxins.

Recently, two groups (20,21) have reported sequence information about the type B toxin, from two different type B strains. Comparable information was already available for the other types of BTX and for tetanus toxin. A comparison of the type B sequence with the sequence of these other toxins indicates that the heavy chain of type B toxin produced by the Danish strain has a sequence homology of 48% to the heavy chain of type A toxin and 35% homology to the equivalent portion of the tetanus toxin sequence. A hydrophilicity plot comparison of various strains of BTX types and of tetanus toxin suggests that for all of them, there is a conserved hydrophobic region in the heavy chain. This region may be important for translocation of the toxin across the extracellular membrane (see below). In contrast, the portion of the toxins that has previously been hypothesized to be most important to receptor binding, the heavy chain carboxyl-terminal end, is one of the regions of most divergent sequence. The sequence difference in this portion of the toxins could explain the specificity of receptor binding reported for the various types of clostridial toxins.

The light chain of the type B toxin produced by the Danish and Okra strains have 31–32% homology to the light chain of type A toxin as reported by these two groups (20,21). Surprisingly, the type B toxin was also found to have a 50–52% homology to the equivalent portion of the tetanus toxin sequence. The light chains of type A, C1, and D BTXs have a lower (roughly 30%) homology to the equivalent portion of the tetanus toxin. Analysis of the light chain sequences of the various types of BTX and of tetanus toxin also indicates a highly conserved region that contains a histidine-rich sequence. This sequence is typical of the active site of metalloproteinases, and it is tempting to assume that such activity could have some role in the intracellular action of the toxins.

PHARMACOLOGY AND TOXICOLOGY OF BOTULINUM TOXIN TYPE B

Mode of Action

Botulinum toxins, including type B toxin, probably act through a three-step process: extracellular binding onto the neuron, internalization, and intracellular poisoning (22). Binding appears to occur as a result of an interaction with specific acceptors on the surface of the presynaptic motor nerve terminal membrane (23). Using radiolabeled toxins and various in vitro/ex vivo models, distinct acceptor sites on the presynaptic terminal for some of the toxins have been found. Using rat cerebrocortical synaptosomes, sites specific for types A, B, E, and F have been reported: the sites are saturable, and toxins of one type bind weakly, if at all, to the acceptors of another type (24). In studies using mouse hemidiaphragms, type B toxin was not found to affect type A binding at all. (However, Black and Dolly reported that a large excess of type A toxin slightly reduced type B toxin binding to mouse hemidiaphragms in vitro [25]). Evaluating binding affinities with rat brain synaptosomes, Evans et al. found that there are two populations of acceptors for type B toxin: a smaller number of high-affinity sites (K_D = 0.3–0.5 nM; B_{max} about 30–60 fmol/mg protein), and a larger number of low-affinity sites (K_D = 16–21 nM; B_{max} > > 3000 fmol/mg protein) (26). In mouse hemidiaphragms, the total density of acceptors for type B toxin (627 ± 21% sites/μm^2) is approximately four times that of acceptors for type A toxin (152 ± 20% sites/μm^2) by electron microscope autoradiography (25).

Binding of the various BTXs to neurons is mediated by the carbonyl end of their heavy chain (27). Both the single-chain unnicked toxin and the heavy chain of type B toxin bind to rat brain synaptosomes, and the heavy chain is a potent inhibitor of the binding of the single chain, whereas the light chain is much less effective (28). Preincubation with the heavy chain of type B toxin antagonizes the in vitro paralysis of mouse hemidiaphragm induced by the active type B dichain (29).

Botulinum toxin binding to the presynaptic nerve membrane may involve both membrane determinants containing sialic acid (probably gangliosides) and one or more proteins (acceptor/receptor proteins) on the neuronal surface membrane (30). The acceptors for BTXs have not yet been isolated or characterized, and the evidence for this double receptor hypothesis is indirect, particularly for the role of gangliosides. Gangliosides, but not other membrane lipids, inactivate BTX in vitro and in vivo (31). Certain lectins with affinity for sialic acid–containing sugars reduce the binding of BTXs to brain membranes and reduce the neuromuscular blocking activity of the toxins (32). The specific gangliosides involved may differ between the various types of BTX. Type A toxin binds avidly to GQ_{1b} gangliosides, whereas type B toxin binds less efficiently to GQ_{1b} ganglioside (33), and more efficiently to GD_{1a} and GT_{1b} gangliosides than type A toxin (34). The results of these studies are highly dependent on the pH and ionic strength of the medium, however. Less is known about the protein component of BTX binding to neurons. It is presumed that there is such a component, and that it has functional significance, by analogy to other, better-characterized receptors. Also, toxin binding to synaptosomal membranes is affected by pretreatment of the membranes with either neuraminidase (attacking the sialic acid residues of the gangliosides) or proteases (35). This combination of neuronal-specific phospholipid and receptor protein requirements may help explain the very specific neuronal affinity of the botulinum toxins.

Binding of the toxin to its acceptor is neither sufficient nor, under experimental conditions, necessary to cause paralysis. Intracellular toxicity of the toxins can be

observed using methods that bypass the binding step. Such methods include intracellular injection of the toxin into *Aplysia* ganglia (36), or using permeabilized cells (37). Similarly, there appears to be a time lag between in vitro binding of the toxin, when toxin antibody is protective, and subsequent paralysis (38).

A number of studies, including the histological studies cited above, have shown that the toxin is internalized after binding to the cell surface. For example, in vitro kinetic studies using mouse hemidiaphragm and antibodies to BTX revealed that after a while, toxin bound to the cell surface became inaccessible to the antibodies. The toxin is therefore presumably internalized (thus inaccessible) before the onset of paralysis (39). More direct evidence was provided by electron microscopy autoradiography, which showed that the toxin appeared to be internalized, in vacuole-like structures (25).

The internalization process is not completely understood, but, like binding, seems to require the presence of the heavy chain of the toxin. After binding, the toxin/receptor complex is taken into an endosome by an active, temperature-dependent process, and the toxin then somehow enters into the cytosol. The endosome has a lower pH than the extracellular milieu, and the lower pH appears to induce a conformational change in the toxin. As a result of the conformational change, hydrophobic domains are exposed that interact more extensively with lipids, as demonstrated using a liposome model system (40). It is hypothesized that the highly conserved hydrophobic region of the heavy chain inserts into the neuronal membrane and forms a channel, allowing some or all of the toxin molecule into the cell. At least in the case of the type B toxin, using a planar lipid bilayer model, it appears that at acidic pH, the heavy chain is capable of forming channels (41).

Inhibition of neurotransmitter secretion is probably caused by a site on the light chain (42). The mechanism by which intracellular poisoning is achieved is unknown for BTX-B, but it is generally believed to involve enzymatic activity and not mass action or receptor occupancy effects. This hypothesis is attractive for two reasons. First, since the paralysis induced by minuscule amounts of the toxin lasts for weeks or months, it seems likely that the toxin must act on an intracellular component by some means that is only slowly reversed. Such changes would most likely be catalyzed by an enzyme. Second, an enzymatic mechanism seems likely by extrapolation from the more clearly understood mechanism of action of other bacterial toxins, such as ricin and diphtheria toxin. These toxins have dichain structures and apparent structure/function activities generally similar to those of BTXs. The heavy chains of ricin and diphtheria toxin are also involved in binding and internalization, and the light chain is the portion with intracellular activity. In these cases, the activity has been demonstrated to be enzymatic (43). Botulinum toxins types C1 and D have been shown to ADP-ribosylate a membrane protein in mouse synaptosomes (44), but until recently there was no convincing evidence that enzymatic activity was associated with the other BTX serotypes, including type B. As noted above, there is some recently discovered homology between a segment of the amino acid sequence in the central core of the light chain of the various BTXs and zinc-dependent metalloproteinases. This sequence contains a histidine-rich motif that for the metalloproteases represents a zinc-binding site and part of the active site of the protease. Initial experiments using type A toxin mutants in which the histidine site has been affected have not shown any effect of the mutations on the effectiveness of BTX in *Aplysia* neurons (45). These results therefore do not support the hypothesis that this putative metalloprotease active site is important to BTX intracellular activity. At the same time, chelating agents such as 1,10-phenanthroline, which are capable of stripping zinc, iron, and calcium from proteins, are capable of inactivating BTXs including type B toxin (46).

All of the BTX serotypes prevent both spontaneous and evoked quantal release of acetylcholine from the presynaptic neuromuscular junction. However, the intracellular mechanism by which this effect is achieved appears to be different. A series of studies has been performed that emphasizes these differences, in synaptosomal preparations, in phrenic nerve–hemidiaphragms, and also in a double-poisoning experiment using types A and B toxins on a triangularis sterni nerve-muscle preparation (47). These studies all suggest differences between the toxins in terms of the response to pharmacologic manipulation of calcium ion movement, or to microtubule-dissociating drugs.

The most frequently studied difference between the toxins is reversibility of acetylcholine release inhibition by the aminopyridines. These agents increase impulse-evoked Ca^{2+} influx by blocking presynaptic potassium channels. As a result, neurotransmitter release can take place. Aminopyridines reverse the poisoning induced by types A and E (48), but the effect is greatest with type A toxin. The poisoning induced by B, D, and F is not reversible by aminopyridines. An increase in extracellular calcium or use of a Ca^{2+} ionophore, A23187 (49), or guanidine more effectively reverses the effects of type A than of type B, C, or E toxins (50). When synaptosomes were preincubated with microtubule-dissociating drugs, there was a reduction of type B (but not of type A) toxin inhibition of neurotransmitter release. This effect did not appear to be related to prevention of binding or internalization of the toxin (49). The most probable explanation for these differences is that the toxins act at different steps in the process that results in neurotransmitter release.

Comparative Muscle Paralytic Efficacy

Relatively few studies have been reported evaluating the in vivo muscle paralytic potency of BTX types other than type A. Usually, these studies have been performed using rats, a species apparently particularly resistant to type B toxin (see below), or rat tissues. In a study by Burgen et al. using a rat in vitro phrenic nerve–diaphragm preparation, when the toxin was added to the perfusate of the in vitro system, it took amounts of type B toxin "... about 500 times greater than the amount of type A toxin required to produce a similar rate of paralysis. When, however, the phrenic nerve–diaphragm preparation was obtained from young guinea-pigs (150–200 g.) typical neuromuscular block followed the addition of 2000 units/ml. of either type A or type B toxin. There were no marked differences in the latent period or rate of paralysis between types A and B toxins on this preparation. The guinea pig is known to be susceptible to type B toxin. . ." (51).

Another study, by Sellin et al. (52), found that it required 1200 mouse LD_{50} of type B toxin to prevent measurable evoked potentials in a rat ex vivo nerve-muscle preparation. The difference between these two studies in terms of the effective dose for type B toxin–induced paralysis may be that Sellin et al. administered the toxin subcutaneously, above the tibialis anterior muscle in vivo, then later removed the extensor digitorum longus muscle to measure in vitro electrophysiological effects, while Burgen et al. added the toxin to the perfusate of their in vitro preparation.

In rats, no toxin studied has been found to be as potent as type A. Type E (53) and F (54) toxins have also been studied with the same model, and it was found that the dose and duration of the paralysis induced in rats was less than with type A. Unfortunately, given the species-specificity of the various toxin types, rodent studies must be considered inconclusive with respect to predicting the relative clinical potency of the various types of BTX.

For this reason, we have undertaken to study the paralytic efficacy of BTX-B in normal nonhuman primates. In these studies, the toxin was injected into three muscles: the trapezius, the abductor pollicis brevis (APB) muscle of the thumb, and the extensor digitorum brevis (EDB) muscle of the foot. Doses injected into muscles ranged from 5 to 80 U/muscle (1 U is the dose equivalent to a mouse intraperitoneal LD_{50} dose). Efficacy in these studies was measured electrophysiologically, by a decrease in the peak amplitude of the evoked compound muscle action potential. Botulinum toxin type B was found to paralyze injected muscles effectively. At 2 weeks after the initial injection, there was a reduction of maximal muscle electromyographic (EMG) amplitude of 80% or more in the APB and EDB muscles injected at all of the doses tested, in all subjects. A similar reduction of maximal trapezius muscle EMG amplitude was observed after 2 weeks in 10 of 11 animals injected with doses greater than or equal to 80 U/muscle. Muscles paralyzed by BTX-B recovered over time. All of the muscles injected with doses in the estimated therapeutic range had evoked compound muscle action potentials that could be measured by approximately 3 months after the last injection. This result is in contrast to results obtained in the same animals 2 weeks after the initial injection, when several muscles had no measurable evoked compound muscle action potentials. On the basis of visual estimates of the mass of the three muscles tested (the actual mass of the injected muscles was not determined), it appeared that for any given dose, the smaller the muscle mass (abductor pollicis brevis < extensor digitorum brevis < trapezius), the longer the duration of paralysis. Evoked compound muscle action potentials in the injected APB tended to be lower in each animal at each injected dose than in the trapezius, although the dose of BTX-B injected into the APB was one-fourth of the trapezius dose. Similarly, whereas at the end of the study the evoked compound muscle action potentials in the APB and trapezius muscles in the lowest-efficacy dose group were no longer statistically significantly different from baseline values, the evoked compound muscle action potentials of both muscles in the highest-efficacy dose group were significantly less than at baseline.

Reinjection of recovering muscles with a second dose of BTX-B resulted in a decrease in the evoked compound muscle action potentials measured 2 weeks after administration of the second dose. The response of the muscle injected with the second dose of the drug did not indicate a cumulative effect of the doses: reinjected muscles were neither more completely paralyzed nor longer paralyzed than muscles injected with a single dose of the drug.

For type A toxin, histochemical studies have been made, evaluating the changes that occur after injection of the toxin. Duchen (55) performed a series of such studies, injecting the type A toxin into the calf muscles of mice. Typically, the muscles atrophied, as expected from a functional denervation. Also observed was a sprouting of nerve fibers and a formation of new end plates, which occurred more rapidly in the soleus (a slow-twitch red muscle) than in the gastrocnemius (a fast-twitch white muscle). The sprouting was observed to take place preterminally as well as terminally, and collateral sprouting was also observed. Over time, as the muscle recovered function, the number of these collateral sprouts decreased. Similar sprouting after type A toxin injection has been reported in frogs (56), primates (57), and humans (58).

The mechanism by which the type B toxin muscle paralysis is reversed over time is unknown, but it may be assumed to be similar to recovery after type A–induced paralysis. This conclusion is based on results of a study submitted for publication, cited in a recent review article (59). The review article reports that this study was performed in albino rabbits, evaluating histochemical changes (acetylcholinesterase stain, muscle fiber size,

and ATPase staining) after injection of either BTX-A or a "crude preparation" of type B toxin." The denervation indicated by histochemical staining and fiber size analysis appeared transient and lasted for about 3 months for both type A and B toxins."

Comparative Toxicity Data

Type B toxin is known to be toxic for humans, as noted in the introduction, when ingested in poorly processed foods. In the United States, most reported cases of botulism have been related to either type A or type B toxin: together they accounted for 91% of the cases of known toxin-type botulism reported to the Centers for Disease Control from 1970 to 1979. Type A toxin is responsible for twice as many cases of food botulism as type B (60). Of the two types of human botulism, type A is considered to be the more severe (61). Human oral toxic doses are difficult to estimate from these oral food poisoning reports, however, and the clinically toxic dose, even by the oral route, is unknown.

Typically, it takes 1 to 2 days for the symptoms of botulism to develop. The highest cranial nerves are affected first, causing medial rectus paresis, ptosis, and sluggish pupillary response to light. They are followed by the lower cranial nerves, then the peripheral motor neurons, finally and often fatally including those that innervate the respiratory muscles. Effects on peripheral muscles are not observed in the absence of ophthalmic changes: in one study of an unusually large outbreak of type B botulism (53 people were hospitalized), all of the patients who later experienced respiratory difficulty or peripheral muscle weakness first demonstrated cranial nerve impairment (62). In some cases, patients have signs of autonomic nervous system dysfunction: constipation, distention of the urinary bladder, and decreased salivation and tearing (63). Blood counts, urinalysis, and clinical chemistry values are normal in botulism, unless there are secondary complications (the most frequent of which is pneumonia associated with respiratory muscle paralysis) (64).

A definitive diagnosis of botulism can be made by electromyography (EMG). In muscles weakened by botulism, the amplitude of the compound muscle action potential is reduced in response to a supramaximal stimulation of the nerve (65). In partially paralyzed muscles, the response to a double stimulus shows an incremental response (66). However, in severely paralyzed muscles, the neuromuscular blockade may be so complete that repetitive stimulation may not be effective (67). Other EMG measurements appear to remain normal in botulism patients: in a report of an extensive EMG study made in a single type B botulism patient, the latency of facial nerve and upper limb motor and sensory conduction velocities remained normal (68).

Within- and between-species toxicity data from various sources for the type B toxin are difficult to compare, for several reasons:

1. Different end points (minimum lethal dose versus LD_{50}); reference species used (in some cases, the data are expressed as mouse lethal units, in other cases, as guinea pig lethal units).
2. Different normalizing units: units per milligram total nitrogen, per absorbance at different wavelengths, per milligram protein measured using different assays. (Interconversions between these units of measure are approximations at best.)
3. Probable differences in the purity of the toxin and/or percentage nicked versus unnicked toxin in the test material.
4. Differences in route of administration: intraperitoneal, subcutaneous, intravenous, oral, even inhaled.

However, as reported even in the earliest studies by van Ermengem, there appears to be species-specificity in terms of the relative sensitivity to type B toxin, with guinea pigs more sensitive to type B toxin than rats or rabbits (69).

Route of administration also appears to make a difference, at least in terms of the amount of the drug required for toxicity by various routes of administration. This should not be surprising, as pointed out in a paper by Lamanna (70):

> Poisoning of an animal by any mode of exposure to a toxin, including inhalation, will depend on the outcome of a sequence of four events: first, the capacity of the toxin to escape destruction inherent in the techniques of application; second, the capacity of the toxin to get to and to remain at the site of deposition where effective systemic absorption can take place; third, the capacity of the toxin after absorption to be transported to the primary site of poisoning; and fourth, the capacity of the toxin to resist specific or nonspecific forces of detoxification on route to the sites of action.

In this paper, for type B toxin, the rapidity of death (presumably inversely proportional to the amount of toxin required for overt toxicity) in rabbits appeared to differ significantly depending on the route of administration. The data, taken from an older article (71), are in Table 1. Note that time to death after injection of the toxin by the intraperitoneal route is significantly longer than after intramuscular injection. Intraperitoneal injection is the route typically used with mice to standardize the relative potency of various preparations of BTXs. Despite differences in the route of administration, the symptoms of poisoning were not apparently different. This is also consistent with toxicity data available for other BTXs.

A great difference in the relative toxicity of type A and type B toxins was also seen in the rat, in the study by Burgen et al. described above, where the LD_{50} (intraperitoneal injection) in 200 g rats of the two toxins were found to be approximately as follows: rat LD_{50}, type A toxin = 25 mouse LD_{50}; rat LD_{50}, type B toxin = 10,000 mouse LD_{50}. Sellin et al., in the comparable study, found that doses of type B toxin of 5000 mouse LD_{50}

Table 1 Order of Rapidity of Death of Rabbits by Various Routes of Injections of Botulinum Toxin Type B

	Time of death		
Route	days	hr	min
Intravenous		2	25
Intra-arterial		2	45
Intramuscular		4	30
Intracerebral		4	35
Intrapulmonary		5	0
Occipital		6	10
Subcutaneous		7	45
Eye anterior chamber		8	15
Intraperitoneal		9	5
Intrasciatic		10	53
Intragastric		33	45
Intrarectal	4	9	0

Source: From Legroux, Levarditi and Jéramec, 1945, as cited in Ref. 70.

caused debilitation or death (in contrast, only 20 mouse LD_{50} units of type A toxin caused similar toxicity). In the Lamanna paper, data were also given comparing the sensitivities of guinea pigs to different types BTX administered by various routes, as listed in Table 2. The doses in this table are expressed in mouse intraperitoneal units, in which one unit is the mouse LD_{50} dose by that route of administration. Whereas for BTX-A and BTX-B toxicity by the various routes is of the same order of magnitude, the type E toxin appeared to be much less toxic. Although no information about the bacterial source strains, level of purification, or pretreatment was provided, the difference is presumably due—at least in part—to the amount of nicking in the tested toxins. Type A toxin is always nicked by the organisms, type B may be (depending on whether the source strain was proteolytic or not), and type E toxin is not nicked endogenously.

In another report, using type B toxin crudely purified from a nonproteolytic strain (the toxin was therefore presumably mostly in an unnicked form), another comparison of route of administration versus test species was made (72). The results are summarized in Table 3. The data from this study suggest that the relative oral potency of this toxin preparation may have been much greater than for the toxin preparation cited by Lamanna. This may reflect the amount of nicking of the toxin (by the oral route, the toxin may have become activated in the gastrointestinal tract, while less activation occurred after parenteral administration).

No primate toxicity data for type B toxin were found in the literature, other than a single-animal, single-dose report. In this study, it was reported that for rhesus monkeys, oral administration of 100 guinea pig minimum lethal dose (least amount that will kill a 350 g guinea pig in 96 hours after subcutaneous injection) was lethal within 24 hours.

As part of our studies evaluating the effects of BTX-B in normal nonhuman primates, we also evaluated the potential toxicity of doses 10 to 20 times those that effectively paralyzed muscles. In these studies, the toxin was injected intramuscularly, in doses divided over five different muscles: the trapezius, abductor pollicis brevis, extensor digitorum brevis, gluteus maximus, and biceps femoris. Doses in this phase of the study ranged from total body doses of 120 to 480 U/kg total body weight. Toxicity was evaluated by clinical observations, clinical chemistries, ophthalmoscopic evaluations, electrocardiograms, and electrophysiological measurements: changes in peak evoked compound muscle action potential of muscles not injected with the drug (expected to be reduced if system weakening was caused by the drug), nerve conduction velocities of peripheral motor and sensory nerves (expected to change as a result of distal myelinopathies and axonopathies), and somatosensory evoked potentials to evaluate potential dysfunction

Table 2 Toxicity of Botulinum Toxins for the Guinea Pig by Various Routes

	Mouse intraperitoneal (ip) units		
Type	ip	oral	respiratory
A	5.2	840	141
B	4.8	413	350
E	78.0	456,000	778

Source: From M.A. Cardella and J.V. Jemski, personal communication, as cited in Ref. 70.

Table 3 Relative Susceptibility of Mice, Guinea Pigs, and Rabbits to Type B Toxin Administered Subcutaneously, or Orally, as Multiples of Number of Guinea Pig Minimum Lethal Subcutaneous Doses

	Route of administration	
Species	Subcutaneous	Oral
Mice	0.2	2
Guinea pigs	1	2
Rabbits	20	100

Source: Based on data from Ref. 72.

in the central nervous system. No signs of toxicity were observed, even after injection of a total of 480 U/kg, either when administered in a single dosing or when administered as two separate doses of 240 U/kg given 11 weeks apart. Specifically, there were no signs of autonomic nervous system deficits, ophthalmic changes, difficulty in swallowing, or inability to maintain normal posture, even at the highest dose tested. Electrocardiographic changes initially observed in a preliminary segment of the study were found to be the result of the ketamine and pentobarbital anesthesia regimen, rather than related to the toxin itself. The EMG studies also did not indicate any central or peripheral neuropathy or systemic muscle weakness, even at the highest doses tested. In one of the test groups, a dose of 240 U/kg was reinjected into the same animals less than 3 months after an initial dose of 240 U/kg. Again, in these animals, no signs of toxicity were observed, according to the measures listed above.

CLINICAL USES OF BOTULINUM TOXIN TYPE B

The relative clinical value of BTX-A and BTX-B in the treatment of cervical dystonia, or other movement disorders, is currently unknown. The structural and pharmacologic differences between the types of toxin, summarized above, suggest that they might not be therapeutically equivalent. Such differences, if real, are likely to be demonstrated only after injection into dystonic muscles. However, even assuming no specific therapeutic benefit of one toxin type over the other, type B toxin could still be useful for this indication. Because the two toxins are antigenically different (antibodies to type A do not block the effects of type B toxin, and vice versa, in animals or in vitro models), the two toxins could be used together, or in rotation, thus reducing antigenic presentation of either toxin. This, in turn, could delay, reduce, or prevent the development of resistance to toxin therapy. In those patients who have already developed resistance to botulinum toxin type A, the type B toxin could provide the best treatment available.

While our studies in nonhuman primates suggest that BTX-B may be effective and have an excellent therapeutic index, the actual effectiveness of this drug can be judged only after use in dystonia patients. The extrapolation of these results from normal nonhuman primates to dystonic humans assumes no major difference in terms of sensitivity to this specific serotype of the toxin, and no unique difference in muscle responsiveness associated with dystonia as opposed to normal muscles.

SUMMARY

The type B botulinum toxin has many biochemical and biological similarities to other serotypes of BTX. At the same time, there are some indications that the type B toxin may have some unique properties that could have beneficial clinical implications. Most obvious of these is that its amino acid sequence is sufficiently different from those of other serotypes that type A–resistant patients may respond to type B. The mechanistic differences in the mode of action of the various types of BTX have not yet been intensively studied under conditions that will allow prediction of clinical benefit (if any) to dystonia patients. Most of these studies to date have been performed in normal rodents or using in vitro/ex vivo models, which, because of interspecies differences in sensitivity to various types of the toxin, may not be directly applicable. Preliminary data from our laboratory, however, suggests that type B toxin effectively paralyzes nonhuman primate muscle after intramuscular injection and is possibly less likely to cause systemic toxicity than type A toxin.

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Clinical characteristics of infant botulism in the United States: a study of the non-California cases

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We reviewed the clinical features of 99 cases of infant botulism reported to the Centers for Disease Control from states other than California for the period 1976 to 1980. There were no toxin-specific differences in the distribution of ages at onset or sex of the cases. For 76 (76%) patients for whom data were available the most common presenting symptoms were poor feeding (43%) and constipation (24%). Weak suck, poor head control, floppiness, weakness in extremities, difficulty swallowing, altered cry and constipation were reported in over three-fourths of the infants for whom data were available. Loss of facial expression, extraocular muscle paralysis, dilated pupils and depression of deep tendon reflexes occurred significantly more frequently among infants with type B botulism than among those with type A botulism. Ventilatory assistance was required for 61% of infants receiving aminoglycosides after the onset of weakness compared to only 26% of those infants not receiving aminoglycosides ($P = 0.01$). Infant botulism presents a characteristic clinical picture and should be suspected when an infant presents with weakness.

INTRODUCTION

Infant botulism is a distinct clinical entity occurring in children generally less than 6 months old and caused by the intraintestinal production of a neurotoxin by *Clostridium botulinum*. The described clinical features of this illness[1-5] have been based on either small case series or cases reported from a limited geographic area. Since the report of the first case in 1976, the Centers for Disease Control (CDC) have collected both clinical and epidemiologic data on the infant botulism. In this study we present clinical data on those cases reported to the CDC between 1976 and 1980 from states other than California, with data on foods eaten prior to onset of illness, presenting signs and symptoms, clinical course and outcome. The epidemiologic features of these cases are reported elsewhere (J. Morris, J. Snyder, R. Wilson, et al. Epidemiologic characteristics of infant botulism in the United States: A study on non-California cases. Submitted for publication to *Journal of Infectious Diseases*.). We show toxin-specific differences in the relative frequency of signs and symptoms of infant botulism and confirm the association of aminoglycoside therapy and the worsening of respiratory function as measured by the need for ventilatory assistance.

MATERIALS AND METHODS

We defined a case of infant botulism as illness in an infant (<1 year of age) with the known effects of botulinal toxin; all cases were laboratory-confirmed by finding either botulinal toxin or *C. botulinum* in a stool specimen. Data concerning infections in asymptomatic cases and data from cases in California were excluded.

Ninety-nine (53%) of the 188 cases of infant botulism reported to the CDC for the period 1976 to 1980 were from states other than California. Twenty-three (23%) cases were from Pennsylvania and 20 (20%) cases were from Utah. All but one Pennsylvania case were type B botulism while all but two Utah cases were type A botulism.

A questionnaire was completed for each case by the local health department or CDC personnel at the time of the patient's illness. The questions concerned toxin type, age at onset (defined as the age at which the infant was first brought to a physician), sex, place of residence, presenting signs and symptoms, clinical course (including need for respiratory support, antimicrobials administered, diagnostic studies performed and outcome. A food preference history was recorded for the period prior to the onset of illness. Formula and breast feeding frequencies were recorded semi-

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quantitatively. The categories used were "exclusively breast-fed," "greater than 50% breast-fed," "fed breast milk and formula equally," "greater than 50% formula-fed," and "exclusively formula-fed."

RESULTS

There were no significant toxin-specific differences in the distribution of ages at onset or sex of cases (Table 1). Questionnaire data, sometimes incomplete, for 90 (91%) of these cases were available for analysis. For 76 patients where data were available, the most common presenting symptoms were poor feeding (43%) and constipation (24%). Irritability (8%), altered cry (8%) and generalized weakness (7%) were less frequently reported.

Symptoms affecting a majority of patients for whom information was available are shown in Table 2. Less commonly reported signs included sluggishly reactive pupils (31/62, 50%), dehydration (28/62, 45%), extraocular muscle paralysis (18/60, 30%), fever (19/74, 26%) and pneumonia (18/71, 25%).

There were no statistically significant associations found with breast or bottle feeding and duration of hospitalization or outcome. Stratification by toxin type did not alter these results.

There were toxin-specific differences in the frequency of certain signs and symptoms occurring during the illness (Table 3). Loss of facial expression, extraocular muscle paralysis, dilated pupils and depression of deep tendon reflexes were observed more commonly among infants with type B than among those with type A botulism. There was no significant difference in the mean length of hospital stay for infants with type A botulism when compared to those infants with type B botulism (39.6 + 31.1 days for infants with type A botulism *versus* 28.4 ± 23 days for infants with type B botulism, $p = 0.08$ (Mann-Whitney U test)). Toxin types was also not found to be associated with the need for ventilatory assistance or with survival.

Thirty-seven (43%) of 86 patients for whom data were available required ventilatory assistance (i.e. mechanically assisted ventilation). There was no association between exposure to formula, cow's milk (pasteurized and unpasteurized), fruit juices, syrup, honey, fruits, vegetables, home-canned foods or baby foods and the need for assisted ventilation. There was also no association between use of assisted ventilation and age at onset of illness, toxin type or presenting signs or symptoms. The use of ventilatory assistance was, however, associated with the administration of aminoglycosides to the patient after the onset of muscle paralysis (Table 4). No association was found between the use of any antimicrobial in the 2 weeks prior to onset of symptoms and the subsequent use of assisted ventilation.

Spinal fluid examination results were available for 75 (83%) of 90 patients. The median number of leukocytes present was 1 (range, 0 to 16) per mm^3 of spinal fluid, and the median spinal fluid protein concentration was 34 mg per dl (range, 10 to 94 mg per dl). "Brief duration, small amplitude, overabundant,

TABLE 1

Age at presentation of infant botulism by toxin type

Toxin Type	No. of Patients of Following Age at Presentation (Weeks)			Sex	
	0–5	6–11	12+	Male	Female
A	4	19	31	30	24
B	6	18	19	20	23
Other[a]	2	0	0	1	1

[a] Includes one patient with type F and one patient with type B/F botulism.

TABLE 2

Common signs and symptoms found in patients with infant botulism

	No. Affected	Total	%
Symptom			
Weak suck	73	76	96
Poor head control	76	79	96
"Floppy" infant	69	72	96
Weakness in extremities	66	71	93
Trouble swallowing	65	71	92
Altered cry	65	73	89
Constipation	63	76	83
Somnolent	44	62	71
Loss of facial expression	42	61	69
Irritable	40	66	61
Sign			
Depressed knee tendon reflexes	30	58	52
Sluggishly reactive pupils	31	62	50

TABLE 3

Comparisons of frequency of signs and symptoms by toxin type

Sign or Symptom	No. Affected/Total		
	Type A toxin	Type B toxin	*P*
Loss of facial expression	12/25 (48)[a]	30/36 (83)	0.004
Depressed knee deep tendon reflexes	7/24 (29)	23/34 (79)	0.005
Dilated pupils	1/23 (4)	9/33 (27)	0.028
Extraocular muscle paralysis	4/26 (15)	14/33 (42)	0.047

[a] Numbers in parentheses, percentage.

TABLE 4

Need for ventilatory assistance and the use of aminoglycosides
Chi square = 5.86; $P = 0.01$; relative risk = 2.23.

Ventilatory Assistance Required	Received Aminoglycosides	Did Not Receive Aminoglycosides
Yes	17 (61)[a]	12
No	10 (26)	28

[a] Numbers in parentheses, percentage.

motor unit action potentials" was noted in 15 (68%) of 22 patients tested.

Follow-up was available for 89 infants; there were three (3%) deaths attributable to infant botulism among these patients. Duration of hospital stay was available for 69 (80%) of the 86 known survivors. The median length of hospital stay was 27 days (range, 2 to 150 days).

DISCUSSION

The interpretation of this study must be tempered by the shortcomings of case recognition and reporting. Without a high index of suspicion and the collection of appropriate specimens, infants with the disease will be missed. Since mild or inapparent cases are missed, observations on the relative frequency of symptoms, risk factors and outcome may be skewed. There were also data missing on many of the reported cases. Despite these limitations our data allowed us to draw some conclusions concerning the clinical features of infant botulism.

The signs and symptoms noted in our series are similar to those previously reported.[1-4] As reported by Arnon and Chin,[4] although constipation was almost always confirmed it was not usually the chief complaint. Poor feeding was the complaint that most frequently led parents to seek medical attention for the infant. Neuromuscular involvement was most frequently manifested by poor head control, floppiness, and difficulty in swallowing, while extraocular muscle paralysis was less frequently noted than in adults with foodborn botulism.[6] Cranial nerve involvement was usually manifest as "loss of facial expression" of "mask-like faces." Hughes et al.[6] in reviewing the data concerning the toxin-specific signs and symptoms of foodborne botulism found that disease due to toxin from type A organisms was more likely to be associated with an increased frequency of dysarthria, blurred vision, dyspnea, diarrhea, sore throat and dizziness. Objective findings, including upper extremity weakness, ptosis, extraocular muscle paralysis, facial nerve paralysis, tongue weakness and nystagmus, were also more frequent among patients with intoxication by type A organisms. In contrast we observed that infants with type B botulism had more frequent findings of neurologic involvement. We cannot explain these apparently contradictory results. Perhaps there are age-dependent differences in the relative neurologic effects of these toxins. An alternative hypothesis is that detection biases led to the identification of a relatively greater proportion of minimally affected infants with type A botulism. We observed a longer duration of hospitalization for type A cases as compared to type B cases as had been reported by Arnon and Chin.[4] This difference, however, was not statistically significant. As Arnon and Chin stated, the overlap in the length of hospital stay for their two groups made these data of limited usefulness.

We have indirectly confirmed the observation that aminoglycoside therapy potentiates neuromuscular blockade of botulinal toxin.[7] The increased need for mechanically assisted ventilation in patients receiving aminoglycoside therapy strongly supports the recommendation that these agents not be used in patients with infant botulism.

Finally we observed only three deaths in our case series. With early recognition and good supportive care, infants with this illness can be expected to recover fully. The clinical features of infant botulism are sufficiently characteristic to allow the clinician to make the clinical diagnosis and obtain appropriate diagnostic specimens. With increasing physician awareness of this disorder more children should be recognized and appropriately managed.

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For a related item, see p. 158.

4. Antimicrobial prophylaxis is not recommended.
5. Systemically administered steroids are not helpful and they are possibly harmful.

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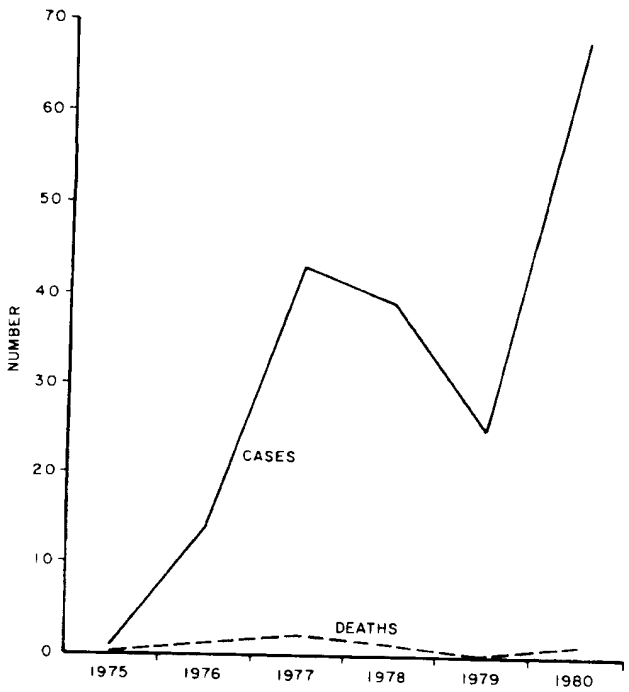
BOTULISM (INFANT)--REPORTED CASES AND DEATHS BY YEAR, UNITED STATES, 1975-1980



Infant Botulism in 1980

In 1980, 68 cases of infant botulism were reported to the Centers for Disease Control. There were 36 males and 32 females, ranging in age from 3 to 38 weeks. Thirty-three cases were due to type A toxin, and 35 were due to type B toxin. There was one fatality. In general type A infant botulism cases occurred west of the Mississippi while type B was distributed throughout the U.S. There has been no marked seasonal variation.

Thirty of the 1980 cases of infant botulism were from one state, California, which has active surveillance and recruits cases for an ongoing study of the clinical and epidemiological features of infant botulism.

During the first 11 months of 1981, 64 cases and 2 deaths were reported. With increased recognition and reporting of this disease, the reported incidence may continue to increase. Infant botulism should be considered in the differential diagnosis of infants less than 6 months old with weakness, constipation, hypotonia and cranial nerve palsies.

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Botulinum B Toxin as an Alternative to Botulinum A Toxin: A Histologic Study

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Summary: Histochemical effects of botulinum B toxin were studied on fibers from longissimus dorsi muscle in Albino rabbits and compared to effects produced by botulinum A toxin. Acetylcholinesterase staining, muscle fiber size analysis, and ATPase staining indicated botulinum B toxin produced a denervation gradient and field similar to that produced by botulinum A toxin. At 5 weeks postinjection with botulinum B toxin, analysis showed muscle fiber size variability, and diffuse acetylcholinesterase fiber staining comparable to botulinum A toxin at the injection site. Muscle sections taken at 4.0 cm for analysis showed statistically significant decreased fiber size variability and contraction of acetylcholinesterase staining pattern for both immunotypes. In addition, the denervation reflected by histochemical staining and fiber size analysis appeared transient and lasted for approximately 3 months for both immunotypes. These findings suggest botulinum B toxin produces pharmacologic effects on innervation of striated muscle similar to botulinum A toxin. Because immunologic tolerance has been demonstrated after therapeutic botulinum A toxin injections, further clinical studies need to be conducted with other immunotypes of toxin with no cross-reactivity to type A.
Key Words: Movement disorders—Botulinum B toxin—Botulinum A toxin—Animal Studies—Blepharospasm.

Botulinum A toxin has been used in clinical studies to treat a number of segmental movement disorders, including blepharospasm, hemifacial spasm, spasmodic torticollis, spasmodic dysphonia, and regional hand dystonias (1–10).

Botulinum neurotoxins are produced by certain strains of the bacterial species *Clostridium botulinum* and *Clostridium baratii* (11). The toxins are classified into seven serotypes A–G. The botulinum neurotoxins comprise a family of pharmacologically similar poisons that block acetylcholine release from peripheral nerves and cause a flaccid paralysis. Type A botulinum toxin is the serotype currently approved by the FDA for use in clinical practice.

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The essential pharmacologic properties of a point injection of botulinum A toxin to striated muscle are (a) the blockade of acetylcholine release; (b) diminished muscle fiber contractility followed by muscle fiber atrophy, which becomes prominent after several weeks (12–14); and (c) a reversal of denervation and muscle atrophy after 10–15 weeks associated with collateral axonal sprouting and contraction of acetylcholinesterase fiber staining (12–15). The regional denervation effect and sequential reversibility of the therapy has been most useful in modulating treatment of regional movement disorders, such as essential blepharospasm (1–5).

The effects of botulism toxin are temporary, therefore most patients have received treatment with botulinum A toxin on multiple occasions over many years (3,14,16). Repeated injections have led to lack of effectiveness in some patients, and sensiti-

zation to botulinum A toxin protein has been associated with its use in others (17–21). Neutralizing antibodies to botulinum A toxin have been demonstrated using the standard mouse assay, and sensitization appears to be related to frequent injections at higher doses (16–21). Neutralizing antibodies as determined with the mouse assay appears to render the therapy ineffective (17,20,21). Due to sensitization, other immunotypes of botulinum toxin need to be considered for clinical application.

This paper reports the histopathologic effects following botulinum B toxin injection into striated muscle and compares these findings to those produced by botulinum A toxin.

METHODS AND MATERIALS

The following is an outline for culture production botulinum B toxin (18). The B toxin used in these experiments was prepared from CDC culture 68 ("bean strain" [origin British culture collection] NCTC-7273). This organism provided the source for the B toxoid preparation used in formulating the pentavalent vaccine.

The culture media consisted of 15 g of tryptocase (BBL) with 5 g of yeast extract diluted in quantity sufficient to 1,000 ml with normal saline. The pH was adjusted to 7.2 with sodium hydroxide (solution A). Another solution consisting of 20% glucose was autoclaved for 15 min at 121° F (solution B). Ten milliliters of solution A was placed in 200 ml of solution B (solution C). A 24 h culture of the organism was made with CMG media (BBL). Five milliliters of the CMG-botulinum B toxin culture were used to inoculate solution C. Toxicity determinations were made over 3 days: day 1—10,000 Mouse LD 50/ml; day 2—100,000 Mouse LD 50/ml; and day 3—100,000 Mouse LD 50/ml (1 IU = one LD 50 for white mouse).

3 N sulfuric acid was added to the flasks after 3 days, which develops the "mud," a suspension with stable biologic B toxin activity.

This preparation was diluted in normal saline containing 5% glycerin and 5% gelatin in acetate buffer adjusted to pH of 4.7.

Specimens taken from longissimus dorsi of 2–3 kg albino rabbits were immediately placed in cold (4°C) formol-calcium (Baker's solution) and fixed for 6–12 h at 4° C. Muscle specimens were then cryoprotected in gum sucrose solution for 3 h. The muscle was oriented both in cross and longitudinal plane on a specimen chuck in OCT compound (Tissue Tek) and frozen in a cryostat. Cut tissue sections (10 μm) were adhered to gel coated slides, air dried for 2 min, and subsequently stained for acetylcholinesterase activity (Geneser-Jensen and Blackstad, 1971) (22). Enzyme histochemistry for myofibrillary ATPase activity (Brooke, Kaiser, 1969) (22) and NADH activity (Scarpelli, Hess, Pearse, 1958) (22) was also conducted on the specimens. Sections for acetylcholinesterase activity were incubated in a solution containing 13 ml of maleic buffer (1.96 g maleic acid, 0.8 g NaOH, 10.8 ml. 1N NaOH, 200 ml distilled water), 10 mg acetylthiocholine iodide, 2 ml 0.03 M cupric sulfate, 1 ml 0.1 sodium citrate, and 0.5 M potassium ferricyanide for 1 h at 37°C. Contiguous cryostat sections were stained either with hematoxylin and eosin or with Gomori trichrome stain to assess normal tissue morphology.

Alternatively, fresh skeletal muscle tissue was flash frozen in isopentane, and cooled to −160° C using liquid nitrogen. Serial cut sections (10 μm) were stained with hematoxylin and eosin or Gomori trichrome to identify any tissue alterations. Enzyme histochemistry for acetylcholinesterase activity was used to quantify endplate structures and assess for denervation.

Histologic measurements were made with the bioquant II system. Fiber size variation comparisons were generated using standard deviation and variance values counted from at least 200 fiber diameters. Also, an F ratio test was conducted to compare fiber size variability.

RESULTS

Five Weeks After Point Injection of Botulinum B Toxin (Dose = 15 IU/kg)

Using the fiber size variability analysis as an indication of denervation, a marked degree of fiber size variability was demonstrated at the injection site 5 weeks after the injection of botulinum B toxin (fiber size diameter median = 44.4 microns; variance = 493; standard deviation = 22.2) (Fig. 1A). When compared to untreated control values (fiber size diameter average = 37.95, variance = 78.6, standard deviation = 8.9), the fiber size variation was significantly greater than controls (F ratio = 4.32 $P < 0.01$).

When comparing a muscle biopsy 4 cm from the injection point, there appeared to be a significant diminution in fiber size variability (median fiber

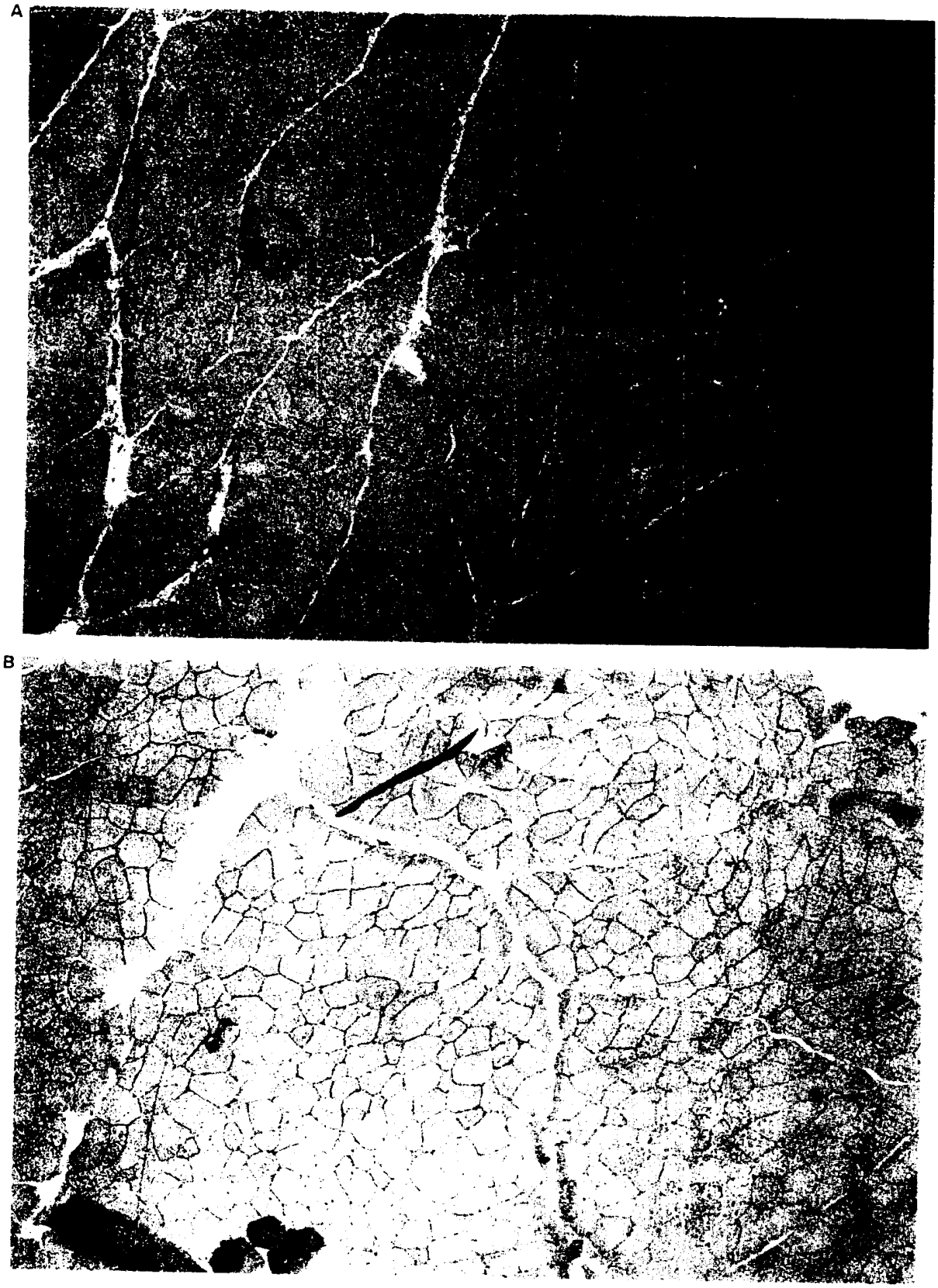

FIG. 1. **A**: Large degree of fiber diameter variability is noted at the injection site (H & E, original magnification ×10). The line on the right hand site of the field is fixation artifact. **B**: Variation in fiber size decreases at increasing distance from the injection site (photo demonstrates fiber cross-section at 40 mm from the injection site, the line on the superior aspect of the field is fixation artifact).

diameter = 58, variance = 278, standard deviation = 16.4) (Fig. 1B). Fiber size variability at 4 cm was significantly different from the injection point (F ratio = 1.77, P < 0.05) indicating regional denervation was more pronounced at the injection site than at 4 cm. Fiber size variability at 4 cm was, however, still significantly greater than fiber variability within control specimens (F ratio = 2.4, p < 0.01) indicating a denervation process even at this distance from the point injection.

Additionally, the spread of cholinesterase was most prominent at the injection site (Fig. 2A). At 4 cm from the injection site, there was a substantial diminution of acetylcholinesterase spread approaching normal intensity (Fig. 2B).

In control specimens, myofibrillary ATPase activity at pH = 9.4 demonstrated type 1 and type 2 fibers. The number or percent ratio of type 1/type 2 fibers was 3.5% of the total. Type 1 fibers were evenly distributed throughout the muscle specimens in the saline injected control tissue. At the injection site there was marked variation of muscle fiber size effecting both fiber types (Fig. 3A). The pattern of fiber typing was altered in that there were now small groups of type 1 fibers, suggesting denervation and renervation. The ratio of type 1/type 2 fibers increased significantly with type 1 fibers representing 22% of the total population. Distally, 4.5 cm away from the injection site, there was much less fiber size variability. The percentage of type 1 fibers was reduced (10.7% of the total), although still not normal (Fig. 3B).

The NADH activity equally demonstrated alterations in the fiber size as well as the fiber typing. In addition, the method identified changes in the intermyofibrillary network consistent with denervation at the injection sites.

Fourteen Weeks After Point Injection of Botulinum Toxin B

There was significantly less acetylcholinesterase staining when comparing the injection site at 14 weeks versus 5 weeks. There were minimal differences in acetylcholinesterase activity at 14 weeks compared to controls. Fiber size variability appeared not to be significantly different from control variability 14 weeks after injection (average diameter = 29.5 microns; variance = 75.7; standard deviation = 8.7; F ratio = 0.7; P = NS).

Furthermore, there was no difference in the fiber size variability or acetylcholinesterase staining pattern when comparing the injection site with muscle tissue 4 cm from the injection after 13 weeks (fiber diameter = 28.1; variance = 54; s = 7.4; F ratio = 0.47, P = NS).

In summary, at 14 weeks both acetylcholinesterase and fiber size analysis did not appear to indicate significant denervation.

Botulinum A Toxin Diffusion Gradient Data After 5 Weeks (Dose = 2–3 IU/kg)

There was considerable fiber size variation at the site of injection associated with the spread of acetylcholinesterase staining on muscle fibers in three animals studied (median diameter = 27.3 microns; s = 14.55; v = 212; F ratio = 2.5; P < 0.01). At 15 mm from the injection site, similar fiber size variability and cholinesterase spread were noted (median diameter = 30.7; s = 12.9; v = 166; F = 1.98, p < 0.01). At 40 mm there was considerable contraction of the acetylcholinesterase staining pattern as well as more uniform muscle fiber diameter sizes (median diameter = 24.9; s = 9.7; v = 93; F = 1.11; P = NS). At 45 mm, the acetylcholinesterase staining pattern and muscle fiber size variations were similar to controls (median diameter = 30.6; s = 6.4; v = 41; F = 0.49; P = NS).

At the same saline injection site and 15 mm intervals, Table 1 outlines control values for fiber size variability and acetylcholinesterase staining pattern.

DISCUSSION

Basic Science and Pharmacology of Botulinum B Toxin Compared to Botulinum A Toxin

Botulinum type A and B neurotoxins exhibit a number of functional, structural, and mechanistic similarities. Both produce chemical denervation at the neuromuscular junction that is thought to occur through a three-step process resulting in the irreversible inhibition of normal neurotransmitter release (23,24). Both species have a molecular weight of approximately 150,000 daltons and the active form of the toxin exists as a dichain molecule consisting of a light (Mr ~50,000 DA) and heavy (Mr ~100,000 DA) chain linked by a disulfide bond (25,26).

Despite the general similarities between the A and B toxins, closer examination of these species reveals very significant differences. All of the neu-

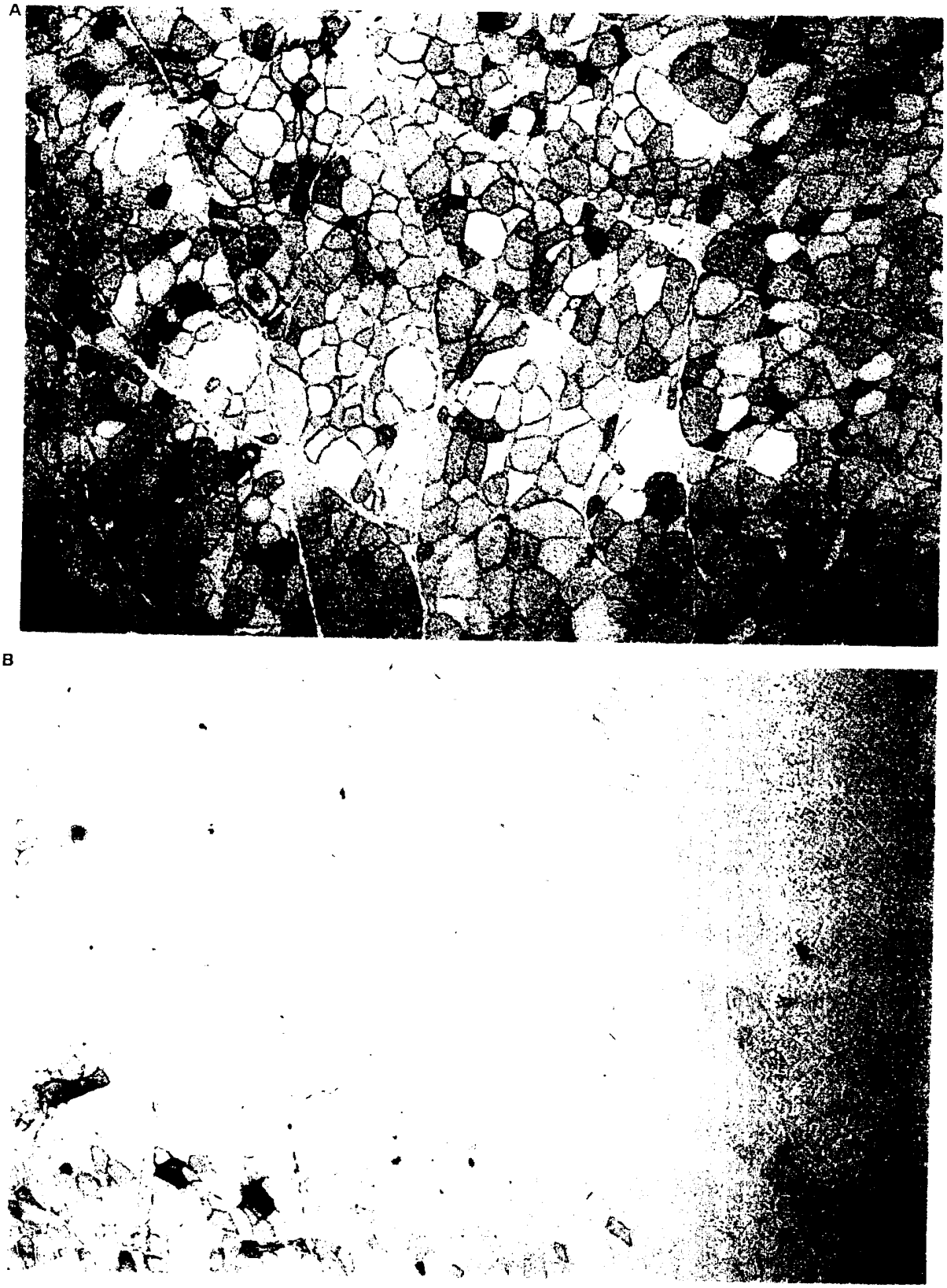

FIG. 2. **A:** Acetylcholinesterase staining pattern is diffuse at the injection associated with high degree of fiber size variability. Both histologic parameters indicate substantial denervation at the injection site. **B:** At 40 mm from the injection site there is marked decrease in acetylcholinesterase staining pattern associated with decreased fiber size variability. The biologic effects of the point injection of botulinum B toxin have diminished at this distance from the point injection (original magnification × 10).

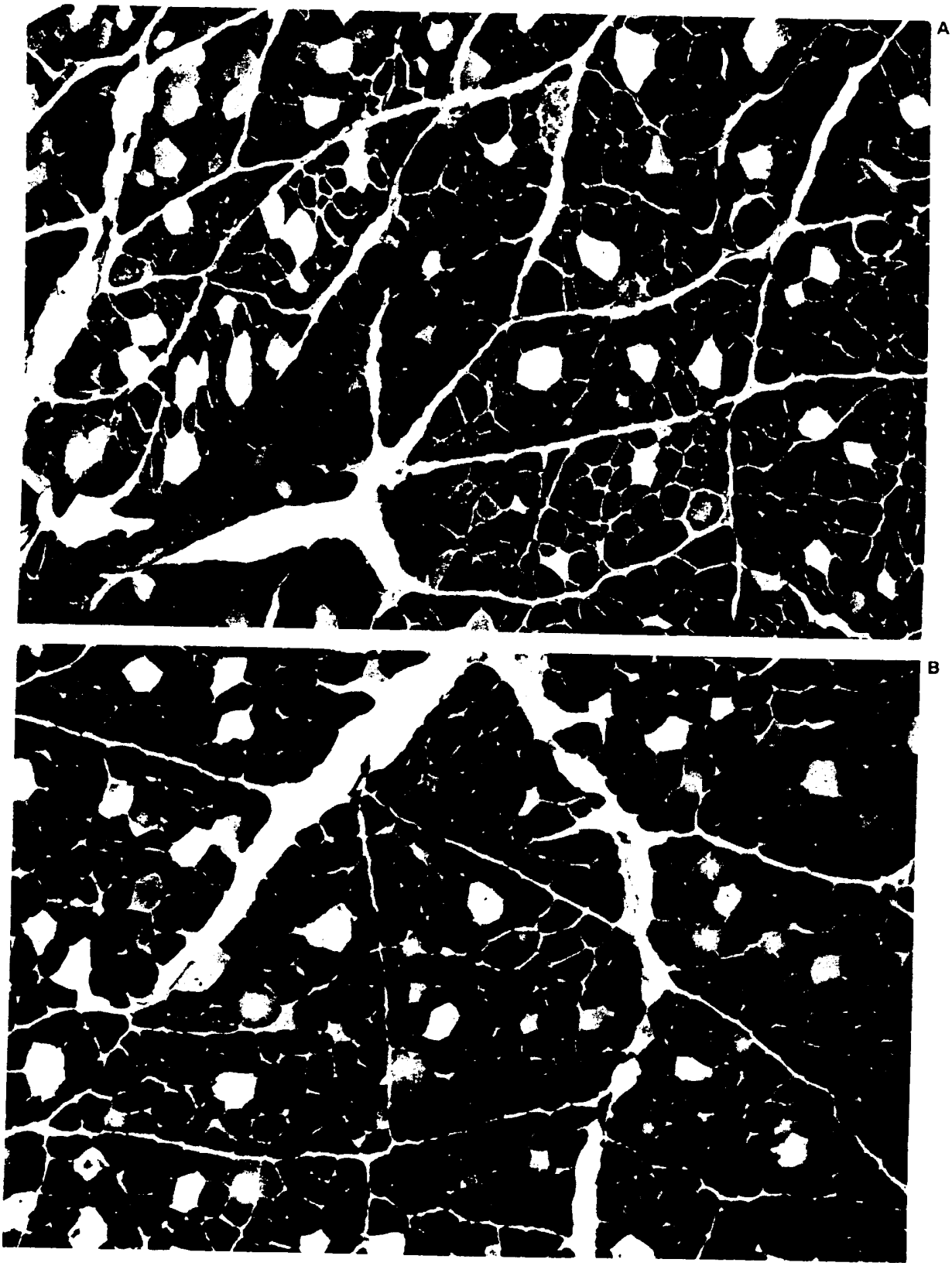

FIG. 3. **A:** ATPase stain at pH 9.4 indicates a predominance of type II fibers. Type I fiber grouping was noted at the injection site compared to the type I fiber distribution at 40 mm from the injection site (**B**) (original magnification ×10). **B:** Type I fibers are evenly distributed throughout the muscle specimen at 40 mm from the injection site.

rotoxins produced by *Clostridium botulinum* are immunologically distinct, which suggests very significant differences in the amino acid sequences of these toxins. Analysis of the partial amino acid sequences for the A and B types has revealed greater homologies between the primary and secondary structure for the light chains than the heavy chains. The degree of primary structure homology is only 20% for the light chains versus 40% for the heavy chains (27). Although similar in secondary and tertiary structure, it is believed that differences in the conformation of the neurotoxins at or near the active site may be responsible for the differences in the neurotoxicity of the toxins (28).

Electrophysiological studies have demonstrated that the botulinum toxins affect different steps in the neurotransmitter release process. Botulinum B toxin affects synchronization of quantal transmitter release whereas botulinum A toxin does not (29). Similarly, differences exist with regard to the reversibility of the inhibition of calcium-dependent release of neurotransmitter. Introduction of calcium into nerve terminals using a calcium ionophore produces the release of transmitter from synaptosomes poisoned by botulinum A toxin more readily than those poisoned by botulinum B toxin (30). At the neuromuscular junction aminopyridine was also more effective at reversal of inhibition produced by botulinum A toxin (31). Ashton and Dolly (30) have recently demonstrated that microtubule-dissociating drugs were effective in blocking the inhibitory effects of botulinum B on neurotransmitter release and ineffective against the A toxin.

The differences in the toxic and neurophysiological effects of the type A and B toxins may be related to the putative existence of two distinct receptor (acceptor) sites for these species (29). Examination of the effect of botulinum A toxin on 125 I-botulinum B toxin binding to neuronal membranes showed a very weak interaction involving both the high and low affinity sites for toxin binding.

Clinical Significance

Botulinum A toxin has been useful in the treatment of regional movement disorders. When injected into muscles, the denervation effect appears to be contained within a definitive field from the injection site, and is temporary in nature.

The diffusion of biologic activity away from the injection site has important consequence to clinical application. Complications associated with therapeutic injection of botulinum toxin in striated muscle appear to result from unwanted spread of the toxin into contiguous muscular structures causing symptomatology. Ptosis and diplopia have been known to occur following eyelid injections, while dysphagia has been associated with botulinum injections to the sternomastoid muscle (15,32). Regional diffusion may also be important for homogeneous effects on an injected muscle. The number and distance between injections may be important in saturating a muscle's innervation zone (33). A fully saturated innervation zone presumably will create a greater denervation effect (33).

TABLE 1. *Control values*

Distance from Point Injection	Fiber diameter (microns)	Variability	Cholinesterase staining pattern
Injection point	37.2	v = 84	focal
15 mm	45	v = 68	focal
30 mm	31.9	v = 35	focal
45 mm	28.5	v = 70.1	focal
60 mm	41	v = 65	focal

The number or percent ratio of type 1/type 2 fibers was 3.5% of the total in control specimens.

The contained region of denervation produced by low dose injections prevents disseminated weakness, a potential complication that may be encountered with very high doses of botulinum A toxin. Containment of biologic effect is, of course, an essential pharmacologic property of therapeutic botulinum injections, which can be assessed by using the histologic analysis outlined in this study. Since regional and reversible denervation are universal effects of therapeutic botulinum A toxin injections for any clinical application, assessing these properties for botulinum B toxin will be important prior to instituting clinical trials with this immunotype. Recently, further pharmacologic investigation with other immunotypes has been cited as a future goal of the botulinum toxin-clinical application technology (National Institute of Health Consensus Conference on Therapeutic Application of Botulinum Toxin, November 1990).

Botulinum B toxin has a different amino acid sequence than botulinum A toxin. As the chemical composition is different than the A toxin, so is the antigenicity of the molecule. It has been long recognized that the cross reactivity of B toxin with A toxin antibodies is extremely small (33–37). Additionally, the receptor site for botulinum B toxin

on the presynaptic membrane is thought to be chemically distinct from the A toxin as has been outlined in the first section of this discussion.

The literature contains reports of negative antibody production in patients treated with botulinum A toxin (1,2). However, other reports show that antibody production is real. In a clinical study performed by Tsui (38), 32 patients with spasmodic torticollis who received repeated injections of botulinum A toxin were tested for the presence of circulating antibodies. The results showed that 4 of 32 patients (12.5%) produced antibodies after 2–9 months of treatment. The remaining 28 patients remained seronegative for up to 30 months. The doses were the same for both the seropositive and the seronegative groups. Other authors have also demonstrated antibody formation in patients treated with botulinum toxin for spasmodic torticollis (20,21). The dose used for the treatment of spasmodic torticollis is usually between 150–300 IU compared to 40–100 IU for blepharospasm. The incidence of antibody formation in blepharospasm patients after multiple botulinum injection over many years still remains unknown, although the work of Gonnering and associates with the assistance of Hathaway (19,39) has suggested this is not a problem in the short term. Recently, a patient with blepharospasm has been shown to develop antibodies to botulinum A toxin after repetitive eyelid injections (unpublished data).

Antibodies have been shown to neutralize the beneficial effect of botulinum toxin in a clinical setting (40). A patient immunized with botulinum A toxoid demonstrated circulating antibodies using the mouse assay. The toxoid was administered for occupational safety purposes. Subsequently, he developed spasmodic dysphonia, and vocal cord injections with botulinum A toxin were attempted. No benefit was obtained presumably because of the presence of circulating neutralizing antibodies (40,41). Antibodies have been demonstrated to occur at botulinum dose levels used to treat blepharospasm in several patients (40–100 IU) (40). More clinical study is clearly needed on the incidence of antibody formation in patients with chronic movement disorders treated with repeated injections of botulinum A toxin over many years to more exactly assess the incidence of sensitization.

Since the B toxin is immunologically distinct and there has been a definite incidence of sensitization occurring with repeated injections of botulinum A toxin, the potential pharmacologic properties of botulinum B toxin were investigated with respect to regional and sequential effects. Additionally, botulinum B toxin may have a different receptor than the A toxin, which could have future therapeutic significance. The method of determining regional denervation that has been helpful for botulinum A toxin has included acetylcholinesterase staining and fiber size variability analysis (15). Upon muscle tissue analysis with acetylcholinesterase histochemistry shortly after injection, patients noted to have had injections of botulinum toxin for blepharospasm were found to demonstrate substantial spread of acetylcholinesterase on muscle fibers (14,15). Furthermore, patients having muscle biopsies within 2 months of botulinum A toxin injection appear to have a higher degree of fiber size variability than controls (13). When biopsies were taken 4 months after injection, cholinesterase staining became indistinguishable from controls and fiber size variability returned to normal (13). The histologic sequence demonstrated on those orbicularis specimens using botulinum A toxin, was reproduced on animal muscle specimens using botulinum B toxin in this study.

In summary, this study has demonstrated that botulinum B toxin is capable of producing regional denervation from a point injection and has a reversibility similar to botulinum A toxin. Therefore, it is anticipated that the regional denervation effect that has been pharmacologically useful in the A toxin will be noted with the application of B toxin.

There may be therapeutic significance to these findings, because immunologic resistance has occurred after repeated injections of botulinum A toxin for the treatment of segmental dystonia. Further studies will be necessary to establish the stability, purity, and biologic activity consistency of botulinum B toxin. Because botulinum B toxin is immunologically distinct, it may have differing biologic effects at the cellular level and may also be useful as adjuvant therapy for patients resistant or refractory to botulinum A toxin.

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